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JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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BIODEPOSIT PRODUCTION BY THE MUSSEL *MYTILUS EDULIS* L. ON ROCKY SHORES[1]

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Marine Biological Station of Asamushi, Tôhoku University, Aomori, Japan

Abstract: Seasonal changes in the amount of biodeposit (faeces and pseudofaeces) produced by the mussel *Mytilus edulis* L., which is one of the representative suspension-feeders in the rocky intertidal and shallow subtidal regions of Mutsu Bay, were studied in the laboratory. The effects of water temperature, light, food concentration, flow rate, body size, age, and spawning on biodeposit production were investigated. More biodeposit was produced in summer than in other seasons. Throughout the year, the amount of biodeposit was positively correlated with body size. Relatively more biodeposit was produced by smaller than by larger individuals. A *M. edulis* population living in one square meter was estimated to produce 9.20 kg of faeces and 2.71 kg of pseudofaeces per year (dry wt). More biodeposit was produced at water temperatures of 17.6–20.2 °C than at 4.5–7.6 °C and 25.2–26.0 °C. The optimum temperature for biodeposit production was found to be ≈ 20.0 °C. When kept in the dark, *M. edulis* produced more biodeposit than in the light. When food concentration is increased, more pseudofaeces are produced; the amount of faeces, however, remains constant. With increasing flow rate, the amount of biodeposit per h increased but the biodeposition rate decreased. Larger amounts of faeces and smaller amounts of pseudofaeces were produced by younger mussels than by older ones of a similar size. Spawning also affected biodeposit production.

INTRODUCTION

Suspension-feeding planktonic and benthic animals filter suspended fine particles and small planktonic organisms, and after either digesting or combining them into aggregates, produce these as faeces or pseudofaeces. Haven & Morales-Alamo (1966) defined this process as biodeposition. Since faeces and pseudofaeces easily sink to the sea bed, the feeding activity of suspension-feeders may influence the physical and chemical environmental conditions and thus the life of benthic organisms. Many studies on biodeposition by oysters have been carried out at oyster farms (Ito & Imai, 1955; Haven & Morales-Alamo, 1966; Frankenberg & Smith, 1967; Arakawa *et al.*, 1971; Tenore & Dunstan, 1973a,b; Kusuki, 1977). They calculated the amount of biodeposit produced by oysters and discussed the importance of biodeposition in the food chain of an aquaculture system. The amounts of biodeposit produced by the pearl oyster *Pinctada martensii* and by the mussels, *Mytilus edulis* and *M. californianus*, have also been estimated (Fox & Coe, 1943; Ota, 1959; Arakawa *et al.*, 1971). The effect of biodeposit on the bottom environment was discussed in these papers.

[1] Contribution from the Marine Biological Station of Asamushi, Tôhoku University, No. 448.

Filtration rates have been the subject of a large number of laboratory studies (see reviews by Jørgensen, 1966; Bayne *et al.*, 1976a; Winter, 1978; Newell, 1979). Most were done in order to determine the effect of environmental factors, such as water temperature, body size, food concentration and flow rate, on filtration rate. In these studies, cultured algae, yeast, and clay minerals were used as food. As biodeposit production and filtration rate are closely interrelated, it is surprising that so few laboratory studies examining biodeposit production have been undertaken. Lund (1957) investigated the relationship between both faecal and pseudofaecal production by the oyster and water temperature under laboratory conditions. Winter (1970) studied the filter-feeding behaviour of *Arctica islandica* and *Modiolus modiolus*. Foster-Smith (1975a, b) investigated changes in the filtration rate and pseudofaecal production with increasing concentration of different particles in suspension, using *Mytilus edulis, Cerastoderma edule* and *Venerupis pullastra*. In these studies however, the relationship between biodeposit production and certain environmental factors was not clarified.

The lower part of the eulittoral zone and the upper part of the infralittoral zone on the rocky coast of Mutsu Bay, northern Japan, are dominated by the mussel *Mytilus edulis* L., which is a representative suspension-feeder in these habitats. Their biomass occasionally amounts to 50 kg (wet wt with shells) per m^2 and amounts to more than 80% of all intertidal organisms (Tsuchiya, 1979). The influence of faeces and pseudofaeces production on the natural habitat cannot, therefore, be ignored.

The present investigation was initiated in October 1977 to measure the seasonal change in biodeposit produced by *M. edulis* in relation to body size. The annual production of biodeposit in the natural habitat can be estimated on the basis of these measured values. The effect of other environmental factors, such as water temperature, light, flow rate, spawning, body size, age, and food concentration, on biodeposit production was also investigated in the laboratory. These basic studies are necessary for an understanding of the effect of this predominant organism on bottom enrichment and the structure and food relationships within intertidal and subtidal communities.

Materials and Methods

Plastic boxes, 24 × 15 cm and 10 cm deep, having a nylon net shelf (2-mm meshes) at a depth of 5 cm, were prepared for measuring of the amount of faeces and pseudofaeces. Mussels used in the experiments were collected from the intertidal rocky shore near the Marine Biological Station of Asamushi, Tôhoku University (40°55′N : 140°50′E). Three individual mussels of 30.31 ± 4.82 g (mean wt ± 90% confidence limit, large size group), five individuals 11.62 ± 0.88 g (medium size group), and 30 individuals 0.83 ± 0.22 g (small size group) were respectively placed on the shelf in three separate boxes. Weight is given as total wet weight including shells. These boxes were hung in a large water tank in which non-filtered natural

sea water was supplied at a flow rate of ≈ 2 l·min^{-1}. Faeces and pseudofaeces deposited on the bottom of the boxes within 24 h were sucked up through a pipette, washed with distilled water and filtered using a Millipore filter (0.45 μm pore size). After desiccation these filters were weighed. The amount of suspended material in the sea water was also measured three times a day by the same method. From October 1977 to October 1978, the amount of faeces and pseudofaeces, suspended materials and water temperature were measured twice a month. Measurements were conducted under natural light conditions.

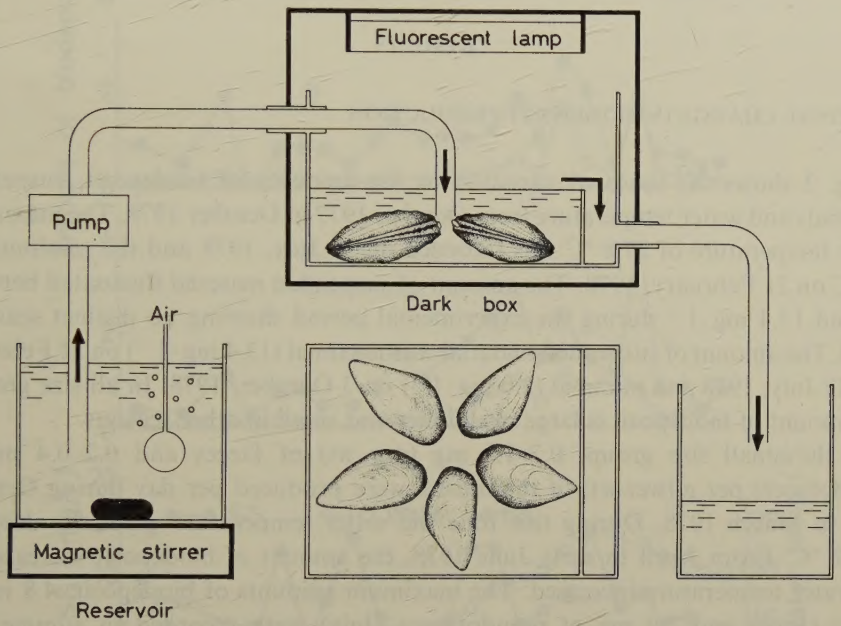

Fig. 1. Experimental apparatus: the water containing food in the reservoir of 10 l is stirred by a magnetic stirrer and supplied to the mussels kept in the plastic box by pump.

The experimental apparatus illustrated in Fig. 1 was designed to determine the effect of certain environmental factors on biodeposit production. Five mussels which had been kept in filtered sea water for 24 h prior to each experiment so that all gut contents had been removed, were arranged in the apparatus so as to enable them to feed equally on the food supplied. Suspended materials deposited on the bottom of the large tank in which natural sea water was overflowing, were used as food in the experiments. The greater part of the food consists of clumps with indefinite physical structure together with planktonic organisms and bacteria. The water containing food in the reservoir was supplied to the mussels by a peristaltic pump for 8 h. After the mussels were seen to open their shells, the water containing the food was supplied. The faeces and pseudofaeces deposited on the bottom of the

boxes were separately removed, dried in the desiccator, and then weighed. Mussels transferred to filtered water after the experiment also produced faeces; the amount was measured by the same method.

In order to determine the amount of biodeposit in the natural habitat, the size distribution and biomass of *M. edulis* were investigated. The *M. edulis* population was sampled within a 10 cm wide transect placed over whole range of the mussel distribution above E.L.W.S. using 10 × 10 cm quadrats. This was done once each season. Seven transects in summer and four in other seasons were surveyed.

RESULTS

SEASONAL CHANGE IN BIODEPOSIT PRODUCTION

Fig. 2 shows the seasonal variation in the amounts of biodeposit, suspended materials and water temperature from October 1977 to October 1978. The maximum water temperature of 27.8 °C was recorded on 25 July, 1978 and the minimum of 3.2 °C on 21 February, 1978. The amount of suspended material fluctuated between 2.0 and 13.4 $mg \cdot l^{-1}$ during the experimental period showing no distinct seasonal trend. The amount of suspended material was maximal (13.4 $mg \cdot l^{-1}$) on 21 February and 25 July, 1978 and minimal (2.0 $mg \cdot l^{-1}$) on 3 October, 1978. In all size groups, the amount of biodeposit is large in summer and small in other seasons.

In the small size group, 0.7–1.8 mg (dry wt) of faeces and 0.2–0.4 mg of pseudofaeces per g (wet wt) of the mussel were produced per day during October 1977 to March 1978. During this time the water temperature gradually dropped to 3.2 °C. From April to early June 1978, the amount of biodeposit increased as the water temperature increased. The maximum amounts of biodeposit, 4.8 mg of faeces (June) and 2.9 mg of pseudofaeces (July) were recorded in summer. In September the amount of biodeposit again decreased. Faeces and pseudofaeces of <2 mg occurred with equal frequency in late autumn, winter and early spring (Fig. 2a).

In the medium size group, the maximum amounts, 2.7 mg of faeces (July) and 1.9 mg of pseudofaeces (Aug.), were recorded in summer and the minimum, 0.1 mg of faeces and 0.1 mg of pseudofaeces, in December (Fig. 2b). In the large size group, the maximum, 3.7 mg of faeces and 1.3 mg of pseudofaeces, was in August and the minimum, 0.3 mg of faeces and 0.1 mg of pseudofaeces, in December (Fig. 2c).

Small mussels produced more biodeposit than the medium and large ones. This difference was more conspicuous in summer than in other seasons, the amount produced by the small size group in summer being more than twice that produced by other groups.

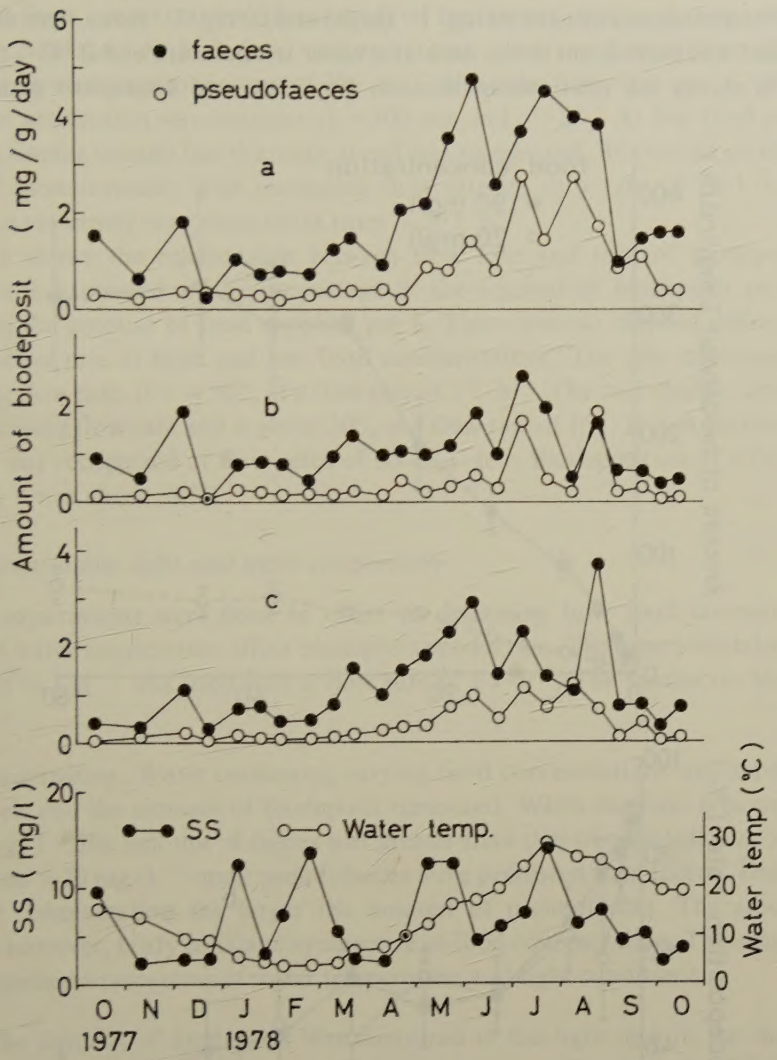

Fig. 2. Seasonal variation in biodeposit (faeces and pseudofaeces), suspended material and water temperature: three size groups of *M. edulis* (a: small, 0.83 ± 0.22 g; mean ± 90% confidence limit. b: medium, 11.62 ± 0.88 g. c: large, 30.31 ± 4.82 g) were used; measurements were conducted at a flow rate of 2 l · min^{-1} under natural light conditions.

FACTORS AFFECTING BIODEPOSIT PRODUCTION

Flow rate

The relationship between flow rate and biodeposit production was studied using the experimental apparatus shown in Fig. 1. Flow rate was controlled by a peristaltic

pump. Food concentrations of 90 mg · l^{-1} (high) and 20 mg · l^{-1} (low) were used. The experiment was carried out in the dark at a water temperature of 8.2 °C.

Fig. 3a shows the relationship between flow rate and biodeposit production.

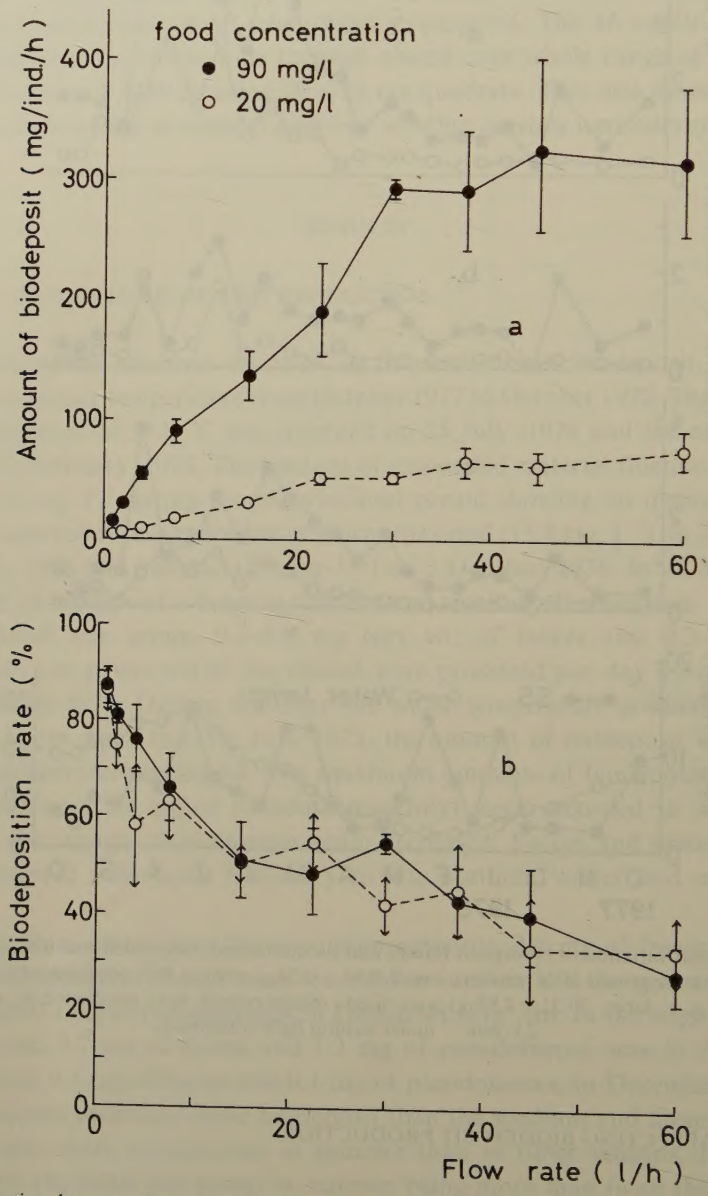

Fig. 3. The relation between flow rate and the amount of biodeposit produced per h (a), and biodeposition rate (b): the latter is calculated as (the amount of biodeposit produced/the total amount of food supplied) × 100; at 8.2 °C in the dark; bars represent SD; mussels of 25.26 ± 5.35 (mean ± 90% confidence limit) g per individual were used.

With both food concentrations the amount of biodeposit produced increased with increasing flow rate. At high food concentrations it increased monotonously with increasing flow rate up to the rate of 30 l · h $^{-1}$. When the flow rate was >30 l · h $^{-1}$, biodeposit production was constant at ≈300 mg · ind. $^{-1}$ · h $^{-1}$. At low food concentrations a similar though less dramatic trend was recognized. Biodeposit production increased monotonously with increasing flow rate up to a rate of 20 l · h $^{-1}$ but showed an extremely slow increase at rates >20 l · h $^{-1}$.

Fig. 3b shows the relationship between flow rate and rate of biodeposition. The latter is expressed as the percentage of the amount of biodeposit produced relative to the amount of food supplied per h. There was no marked difference in biodeposition rate at high and low food concentrations. The rate decreased with increasing flow rate. It is ≈90% at a flow rate of 1 l · h $^{-1}$. The rate sharply decreased with increasing flow rate and is about 50% at a flow rate of 15 l · h $^{-1}$. A further small decrease was recognized at flow rates of 20–60 l · h $^{-1}$. Biodeposition at a flow rate of 60 l · h $^{-1}$ was about 30%.

Food concentration, light, and water temperature

These experiments were done in order to determine how food concentration, light, and water temperature affect biodeposit production. The experimental apparatus shown in Fig. 1 was used with a flow rate of 1 l · h $^{-1}$. The results are shown in Fig. 4.

Food concentration. Water containing varying food concentrations was supplied to the mussels and the amount of biodeposit measured. When the food concentration was 10 mg · l $^{-1}$, the amount of faeces was greater than that of pseudofaeces. At concentrations >20 mg · l $^{-1}$, more pseudofaeces were produced than faeces. The higher the food concentration the larger the amount of pseudofaeces. The amount of faeces is, however, fairly constant irrespective of food concentration. The same trend was recognized irrespective of water temperature and light conditions.

Light. The amount of biodeposit was measured in the light and in the dark. To provide continuous light the experimental apparatus was illuminated at 3000 lux by a fluorescent lamp. In the dark, the amount of pseudofaeces increases monotonously with increasing food concentration. In continuous light, the rate of increase in the amount of pseudofaeces with increasing food concentration at low levels (0–40 mg · l $^{-1}$) is higher than that at high levels (50–60 mg · l $^{-1}$). For instance, when water containing 40–50 mg · l $^{-1}$ of food at 4.5–7.6 °C was supplied, the daily production of pseudofaeces was ≈3 mg · g $^{-1}$ both in the light and the dark. In the case of 100 mg · l $^{-1}$ food supply, about 40 mg · g $^{-1}$ of pseudofaeces was produced per day in continuous light and about 55 mg in the dark. The same trend was seen at 17.6–20.2 °C and 25.2–26.0 °C. There was no significant difference in faecal production between light and dark conditions.

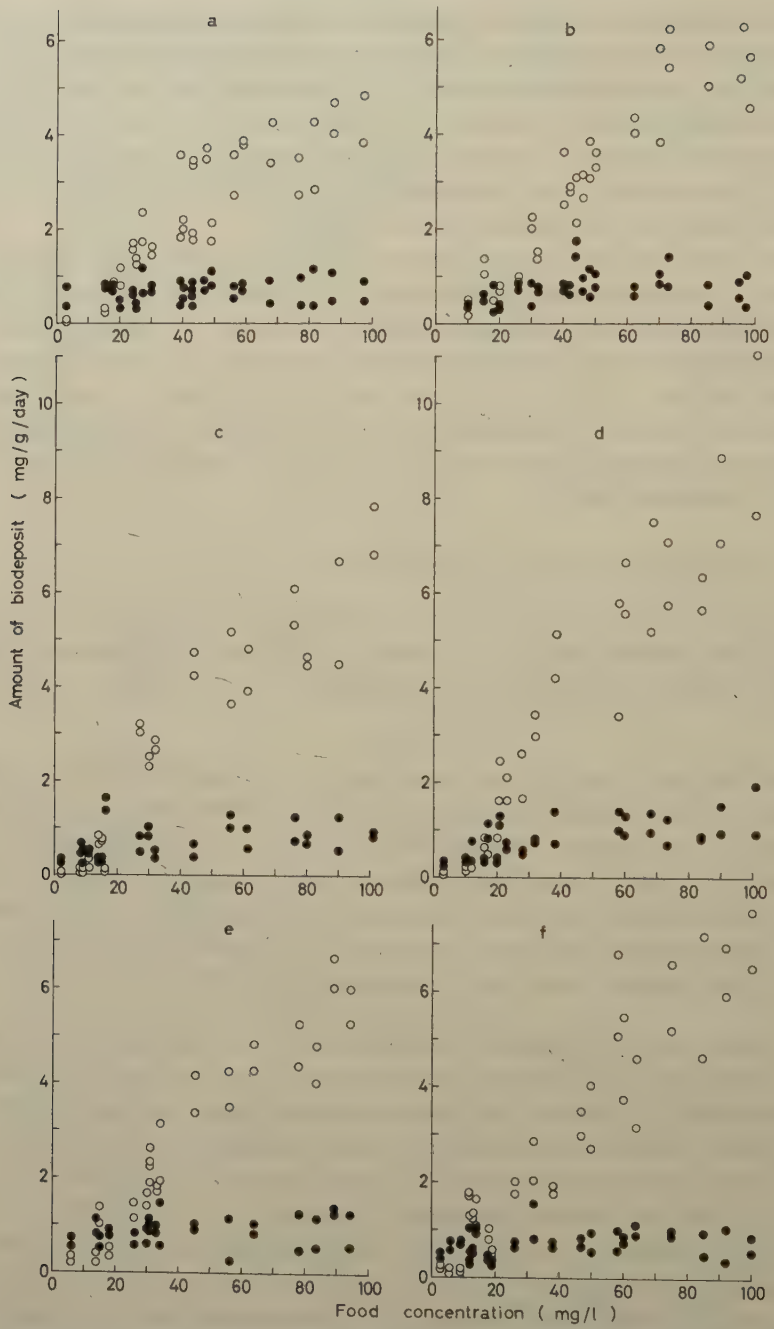

Fig. 4. The relation between food concentration and the amount of biodeposit in the dark and in the light at different water temperatures: ●, amount of faeces; ○, amount of pseudofaeces; a, light at 4.5–7.6 °C; b, dark at 4.5–7.6 °C; c, light at 17.6–20.2 °C; d, dark at 17.6–20.2 °C; e, light at 25.2–26.0 °C; f, dark at 25.2–26.0 °C; mussels of 25.26 ± 5.35 (mean ± 90% confidence limit) g per individual were used.

Water temperature. These experiments were done in different seasons to determine the effect of water temperature on biodeposit production. The amount of pseudofaeces at 17.6–20.2 °C was greater than that at 4.5–7.6 °C. This is more conspicuous at food concentrations >30 mg · l^{-1} and more noticeable for pseudofaecal than for faecal production. The amount of biodeposit at 17.6–20.2 °C was slightly less than that at 25.2–26.0 °C, which is near the maximum water temperature in Mutsu Bay (Tsuchiya, 1979; Tsuchiya *et al.*, 1979).

Body size and age

It has already been shown that small-sized mussels produced more biodeposit per unit body wt per day than the larger ones (Fig. 2). Next, the effects of body size and age on biodeposit production were investigated. Similar sized individuals collected from the scallop rearing cages (Group A, 8 months old), which were submerged at a depth of ≈15 m, and from the intertidal rocks (Group B, 2 yr old) were used to measure faecal and pseudofaecal production rates at water temperatures of 12.3–13.0 °C (Table I). The results of all the experiments show that mussels from

TABLE I

The amount of faeces and pseudofaeces produced by young mussels and by old mussels of similar size (size given as g wet wt including shell): faeces and pseudofaeces given as mg dry wt per g wet wt mussel per day; A, mussels collected from the scallop rearing cages (8 months old); B, mussels collected from the intertidal rocks (2 yr old).

		Size (wt) (g, mean ±90% confidence limit)	Food concentration (mg · l^{-1})	Faeces (mg · g^{-1} · day^{-1})	Pseudofaeces (mg · g^{-1} · day^{-1})	Faeces + pseudofaeces (mg · g^{-1} · day^{-1})
Expt. 1	A	16.1 ± 2.3	35.6	3.74	2.84	6.58
	B	16.4 ± 1.6	35.6	1.50	3.10	4.60
Expt. 2	A	16.1 ± 2.3	51.8	3.43	4.00	7.43
	B	16.4 ± 1.6	51.8	1.20	4.31	5.51
Expt. 3	A	16.1 ± 2.3	34.0	2.89	2.06	4.95
	B	16.4 ± 1.6	34.0	1.68	3.00	4.68
Expt. 4	A	11.3 ± 1.6	40.0	5.18	2.78	7.96
	B	11.6 ± 0.9	40.0	2.36	3.18	5.54
Expt. 5	A	11.3 ± 1.6	69.6	4.86	12.64	17.50
	B	11.6 ± 0.9	69.6	2.75	15.22	17.97
Expt. 6	A	11.3 ± 1.6	28.4	4.12	1.88	6.00
	B	11.6 ± 0.9	28.4	2.87	3.05	5.92

Group A produced more faeces than those from Group B. The opposite trend was recognized for the production of pseudofaeces. The amount of total biodeposit produced by Group A is larger than that by Group B in all cases except Experiment 5.

Spawning

Spawning was occasionally observed during the course of the experiments and continued for 1 or 2 h. A small amount of biodeposit was produced during the period of spawning. In order to determine the amount of biodeposit just after spawning, mussels were forced to spawn by electric stimuli. Five individuals so

TABLE II

The amount of faeces and pseudofaeces produced by the mussels just after spawning: units as in Table I.

Size (wt) (g, mean ±90% confidence limit)	Amount of suspended material ($mg \cdot l^{-1}$)	Faeces ($mg \cdot g^{-1} \cdot day^{-1}$)	Pseudofaeces ($mg \cdot g^{-1} \cdot day^{-1}$)	Faeces + pseudofaeces ($mg \cdot g^{-1} \cdot day^{-1}$)
10.5 ± 1.97	2.0	1.87	0.17	2.04
12.8 ± 2.15	4.6	1.58	0.36	1.94
19.7 ± 3.86	3.5	0.66	0.13	0.79
20.4 ± 4.83	4.1	0.38	0.06	0.44
21.9 ± 8.42	3.4	0.50	0.05	0.55

treated were put in a plastic box in the large tank provided with natural sea water at a flow rate of $\approx 2\ l \cdot min^{-1}$; water temperature was 12.6–14.7 °C. The amount of faeces and pseudofaeces produced in 24 h was measured (Table II). These values did not differ markedly from those shown in Fig. 2.

ANNUAL PRODUCTION OF BIODEPOSIT

Fig. 5 shows the relationship between body weight and the amount of faeces and pseudofaeces produced each season (spring: Mar.–May, summer: June–Aug., autumn: Sept.–Nov., winter: Dec.–Feb.). This figure was drawn using the values shown in Fig. 2. These regression lines show a positive correlation between body size and the amount of biodeposit. These lines can be considered as mean daily production of faeces and pseudofaeces for each size class through each season. The largest amount of biodeposit was produced in summer, and the smallest in winter; the amounts in spring and autumn were comparable to each other.

The size distributions of *M. edulis* on the intertidal rocky shore were investigated for each season (Fig. 6). Smaller individuals are attached at the higher level and the larger at the lower levels throughout all seasons. This is more easily seen in summer than in any other season. In summer, many small individuals attach at the higher level of the mussel's distribution range. The annual production of biodeposit produced by the mussel population living on the intertidal rocky shore was estimated as follows using the values shown in Figs. 5 and 6.

Table III provides an example of how the estimation of daily production of faeces and pseudofaeces produced by *M. edulis* living 30 cm above E.L.W.S. was calculated.

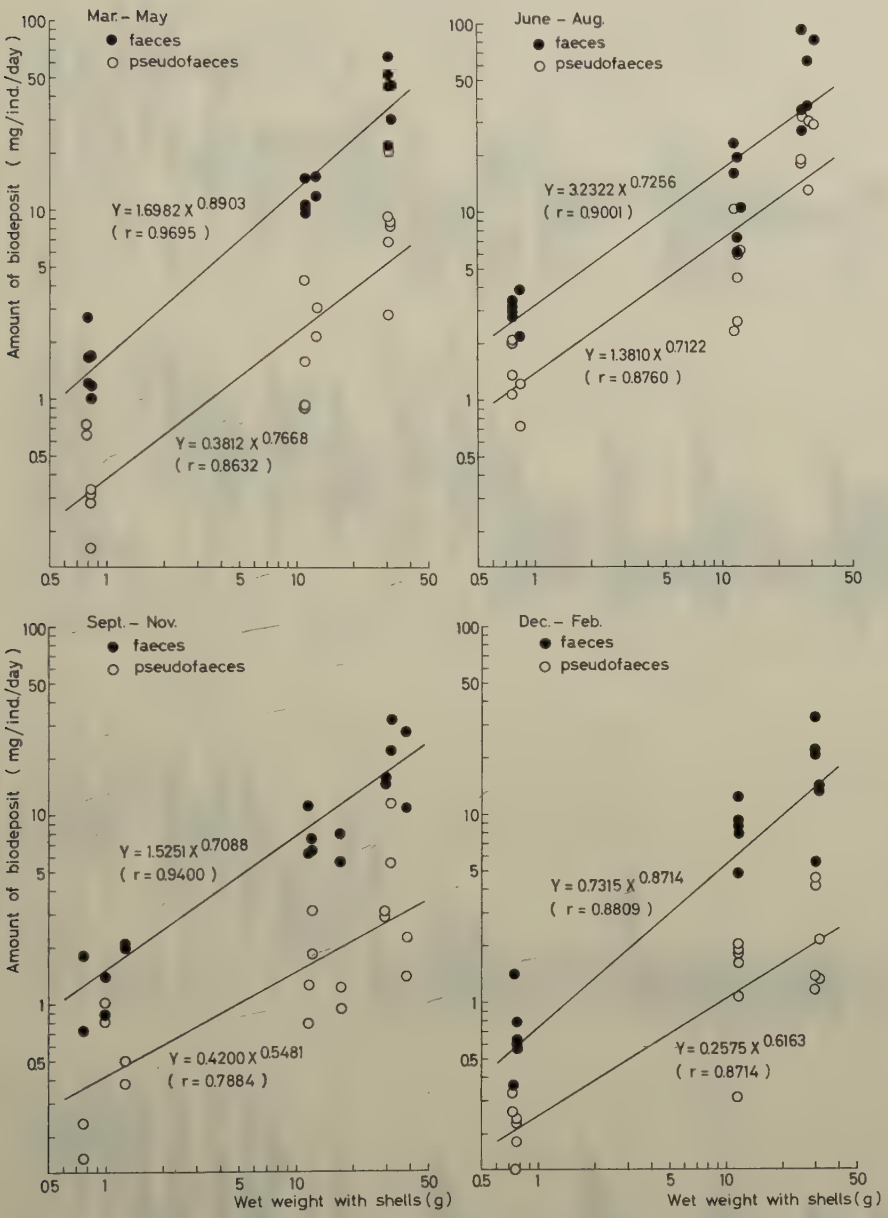

Fig. 5. The relation between body size and the amount of biodeposit for each season: these figures were drawn using the values shown in Fig. 2.

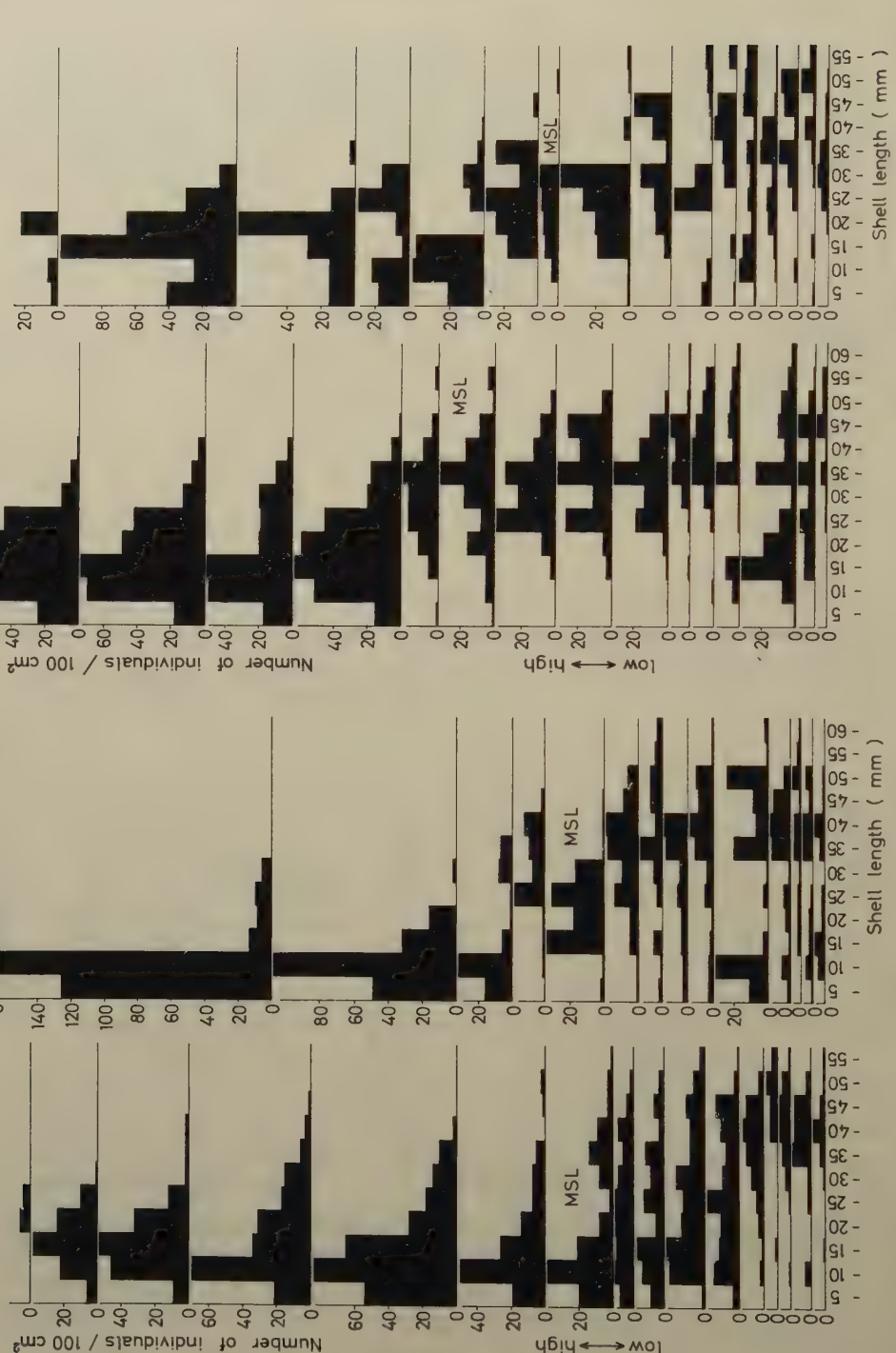

Fig. 6. Size distribution of *M. edulis* collected from the 10 cm wide transect over whole mussel distribution above E.L.W.S. for each season: a, Mar.–May; b, June–Aug.; c, Sept.–Nov.; d, Dec.–Feb.

TABLE III

Daily production of faeces and pseudofaeces produced by *M. edulis* from 30 cm above E.L.W.S. on the spring tide during Mar. to May, 1978: mean submerged time in a day is 17 h; water temperature, 4.0–15.8 °C, amount of suspended material, 2.1–12.4 mg · l^{-1}; weight of biodeposit (faeces and pseudofaeces) is expressed in dry wt.

Shell length (mm)	Mean weight (g wet wt)	No. · 100 cm^{-2}	Faeces (mg · ind.$^{-1}$ · day^{-1})	Pseudofaeces (mg · ind.$^{-1}$ · day^{-1})	Faeces (mg · 100 cm^{-2})	Pseudofaeces (mg · 100 cm^{-2})	Faeces + pseudofaeces (mg · 100 cm^{-2})
0–5	0.09	2.25	0.199	0.060	0.448	0.135	0.583
5–10	0.15	21.25	0.314	0.089	6.673	1.891	8.564
10–15	0.38	23.00	0.718	0.182	16.514	4.186	20.700
15–20	1.01	14.50	1.713	0.384	24.839	5.568	30.407
20–25	2.04	13.00	3.204	0.659	41.652	8.567	50.219
25–30	3.24	17.75	4.836	0.939	85.839	16.667	102.506
30–35	4.48	9.50	6.454	1.204	61.313	11.438	72.751
35–40	6.96	9.00	9.554	1.688	85.986	15.192	101.178
40–45	10.03	11.25	13.227	2.233	148.804	25.121	173.925
45–50	13.97	7.25	17.765	2.879	128.796	20.872	149.668
50–55	18.12	3.75	22.394	3.515	87.978	13.181	97.159
		Total			684.842	122.818	807.660
		× submergence in a day (17/24)			485.096	86.996	572.092

TABLE IV

Daily production of biodeposit by *M. edulis* collected from the 10 cm wide transect over whole range of the mussel distribution above E.L.W.S.

Tide level (cm)	No. · 100 cm^{-2}	Biomass (g wet wt)	Submergence in a day (h)	Faeces (g dry wt · day^{-1})	Pseudofaeces (g dry wt · day^{-1})	Faeces + pseudofaeces (g dry wt · day^{-1})
75	10.00	13.0	4.2	0.004	0.001	0.005
70	102.25	66.7	5.0	0.024	0.005	0.029
65	138.75	114.0	6.7	0.055	0.012	0.067
60	196.75	193.3	8.0	0.102	0.021	0.123
55	281.25	228.3	10.6	0.164	0.035	0.199
50	130.50	129.0	11.9	0.100	0.020	0.120
45	124.00	268.0	12.8	0.202	0.038	0.240
40	81.00	226.3	13.8	0.215	0.040	0.255
35	97.25	242.3	16.4	0.228	0.042	0.270
30	132.50	472.5	17.0	0.485	0.087	0.572
25	108.25	401.5	18.0	0.420	0.076	0.496
20	57.25	305.1	19.9	0.303	0.061	0.364
15	18.00	206.4	21.9	0.247	0.041	0.288
10	28.25	227.3	22.1	0.285	0.049	0.334
5	41.75	272.5	22.5	0.509	0.085	0.594
0 (E.L.W.S.)	25.25	190.3	24.0	0.218	0.038	0.256
Total	1573.00	3556.5		3.561	0.651	4.212

Mussels were collected from the rocky intertidal shore on spring tides during Mar.–May, 1978. The mean daily production of faeces per individual for each size class living at this site was obtained by applying the regression line shown in Fig. 5. The calculated values are the average amounts of faeces and pseudofaeces produced by mussels living at this shore level per day and are calculated on the assumption that they are submerged throughout the day. Mussels living in the intertidal zone are exposed for a limited period each day, and feeding and biodeposit production do not occur during this period. Therefore, the actual amount of biodeposit can be obtained by an appropriate correction. The time that each tidal level was submerged each day was measured several times a year considering the conditions prevailing on the shore (near the Marine Biological Station of Asamushi). In the present example the period of submersion time is 17.0 h · day^{-1}; thus the actual amount of material produced per day is 17/24 of the calculated values. Similar calculations were made for mussels at all levels in the intertidal at Asamushi (Table IV). Here, 3.561 g (dry wt) of faeces and 0.651 g (dry wt) of pseudofaeces per day were estimated to be produced by *M. edulis* in a 10 cm wide transect over the whole range of the mussel distribution above E.L.W.S. on the spring tide in spring.

TABLE V

Daily production of biodeposit by *M. edulis* collected from the 10 cm wide transect over whole range of the mussel distribution above E.L.W.S. at each season.

Season	Tide	Faeces (g dry wt · day^{-1})	Pseudofaeces (g dry wt · day^{-1})	Faeces + pseudofaeces (g dry wt · day^{-1})
Spring	Spring	3.561	0.651	4.212
(Mar.–May)	Neap	3.402	0.616	4.018
Summer	Spring	6.094	2.589	8.683
(June–Aug.)	Neap	6.919	2.927	9.846
Autumn	Spring	4.309	0.930	5.239
(Sept.–Nov.)	Neap	5.106	1.123	6.229
Winter	Spring	1.346	0.311	1.657
(Dec.–Feb.)	Neap	1.531	0.342	1.873

The amount of biodeposit at other seasons was estimated in the same way (Table V). These data suggest that the largest amount of biodeposit is produced in summer and the smallest in winter. There is no marked difference between the amounts produced during spring and neap tides in all seasons.

DISCUSSION

It is most likely that the biodeposit produced by mussels living in the rocky intertidal has some effect on the environmental conditions of coastal water and the sea

bed. In order to approach this problem the amount of biodeposit must be estimated. In the present study, the seasonal variation in the amount of biodeposit of the mussel *M. edulis* was measured in the laboratory for the first time, and the annual production of biodeposit was estimated. As sufficient natural sea water was supplied to the culture system under natural light conditions, the amount of biodeposit measured in this study may give a reasonable approximation of biodeposit production in the natural habitat.

Within a 10 cm wide transect over the entire vertical range of *M. edulis* above E.L.W.S., the daily production of biodeposit on the intertidal rocky shore at Asamushi was estimated to be 1.346–6.919 g (dry wt) of faeces, 0.311–2.927 g (dry wt) of pseudofaeces and hence 1.657–9.846 g (dry wt) of total biodeposits. These calculations take into account the difference in the size distribution of mussels during each season and the prevailing tidal conditions. The mean daily production was calculated to be 4.034 g of faeces, 1.186 of pseudofaeces and hence 5.220 of total biodeposit. The total biodeposit produced is calculated to be 11908.2 $g \cdot m^{-2} \cdot yr^{-1}$ (faeces, 9202.6 $g \cdot m^{-2} \cdot yr^{-1}$; pseudofaeces, 2705.6 $g \cdot m^{-2} \cdot yr^{-1}$). As *M. edulis* attaches not only intertidally but also in large numbers to the subtidal rock surface and scallop rearing cages, the actual amount of biodeposit in this coastal area may be larger than the values estimated in this study. Consequently, it is suggested that the biodeposit produced by *M. edulis* living in the natural habitat has an important effect upon the environmental conditions of the sea bed and coastal waters perhaps in the similar manner to the biodeposit produced by the oysters in mariculture farms.

The amount of biodeposit was largest in summer in all size groups (Fig. 2); it was also larger at 17.6–20.2 °C than at 4.5–7.6 °C irrespective of food concentration. However, the amount of biodeposit was slightly smaller at 25.2–26.0 °C (Fig. 4). This fact suggests that there is an optimum temperature for biodeposit production ($\approx$20 °C). As several workers have reported that filtration rate increases with water temperature up to an optimum after which a relatively sharp decrease occurs (Ali, 1970; Dame, 1972; Widdows, 1973; Schulte, 1975; Bayne *et al.*, 1976b), this may also be the case with biodeposit production. Winter (1970) has reported this phenomenon. Biodeposit production must be greatly influenced by water temperature.

There was no marked seasonal variation in the amount of suspended material during the experimental period. Even when large amounts (>10 $mg \cdot l^{-1}$) were present in the natural sea water, the amount of biodeposit was not always large in all size groups (Fig. 2). The positive relationship between the amount of biodeposit and the amount of suspended material was not recognized in the experiment where natural sea water was supplied. The water generally contained suspended materials <10 $mg \cdot l^{-1}$ and occasionally >10 $mg \cdot l^{-1}$ in this experimental period. Therefore, seasonal variation in the amount of biodeposit may be influenced by water temperature rather than by the amount of suspended material at these low levels.

Under conditions of low food concentration, the mussels produced more faeces

than pseudofaeces; the amount of the latter increased with increasing food concentration, while that of the former was constant irrespective of water temperature and light conditions. Many workers have also recognized a similar trend in biodeposit production (Chiba & Ohshima, 1957; Haven & Morales-Alamo, 1966; Tenore & Dunstan, 1973b; Foster-Smith, 1975a). These observations suggest that when food is present in excess, the mussel utilizes the amount of food needed and eliminates the remainder as pseudofaeces. When suspended materials exceed 20 $mg \cdot l^{-1}$ in the natural habitat, the mussel may certainly produce more biodeposit as shown in Fig. 2. It is well known that filtration rate decreases with increasing food concentration for different species of bivalves (Dral, 1968; Winter, 1970, 1973; Walne, 1972; Schulte, 1975; Epifanio & Ewart, 1977). Consequently, it is speculated that when food is present in abundance, the amount of biodeposit does not increase proportionally to the amount of food supplied. This phenomenon was recently reported by Widdows *et al.* (1979).

Larger mussels produce more biodeposits (Fig. 5), but the amount of biodeposit per unit body weight is greater in smaller individuals than in larger ones (Fig. 2). It is suggested that body size is an important factor in determining the amount of biodeposit. These results seem to be due to the difference in physiological activity that occurs during the growth of *M. edulis*, but details of this process are unknown. These results show the relative reduction of biodeposition activity in larger animals. Many workers have recognized a similar trend in filtration rate in various bivalves (Walne, 1972; Vahl, 1973; Winter, 1973; Foster-Smith, 1975b). These results, however, require circumspection because body size and age are not always parallel. Table I shows that the amount of faeces produced by the younger mussels (wt ≈ 16 and 11 g per individual, 8 months old) is larger than the amount produced by older ones of similar size (2 yr old). The reverse trend is seen with respect to pseudofaecal production. It is suggested that the younger the mussels, the higher the feeding activity.

The amount of water filtered increases with increasing flow rate (Walne, 1972; Hildreth & Crisp, 1976), hence it is likely that the amount of biodeposit also increases with increasing flow rate; Fig. 3 shows this to be the case. It is suggested that there is a limit to the amount of biodeposit aggregated by the mussels. Mussels ingest or combine most of the suspended materials supplied as food at low flow rates. At high flow rates, however, a large fraction of the suspended material does not enter the mussel and they may not be able to combine the whole amount attached to their gills on account of the strong water movement. In this way, the amount of biodeposit produced per h increases with increasing flow rate as shown in Fig. 3. On the other hand, the biodeposition rate decreases with increasing flow rate. Similar rates were recognized at any flow rate irrespective of food concentration. It is suggested that the mussels filter the same volume of water supplied per h in any food concentration.

The amount of pseudofaeces was greater in the dark than in the light (Fig. 4).

This trend was most easily seen at food concentrations of 50 mg·l^{-1} or more and was independent of water temperature. Light has an adverse effect on pseudofaecal production. The level of faecal production was the same in the light or in the dark. Arakawa *et al.* (1971) also reported that more active feeding was recognized at night than in daytime.

An individual *M. edulis* takes 1 or 2 h to spawn (Iwata, 1949). It was observed that the mussels produced very little biodeposit whilst spawning. The usual amount was, however, produced immediately after spawning as feeding activity quickly returns to normal (Table II). Spawning occurs during early December to late May in Mutsu Bay (Hirai, 1963) and is not synchronized. Therefore, it may not have a great influence on the seasonal variation in biodeposit production.

Previously, algal cells or inorganic particles have been used to determine the effects of several factors on the filtration rate. Few studies have been conducted using suspended materials found in natural sea water as in the present study. Recently, a study of feeding activity using seston as food for *M. edulis* has been reported (Widdows *et al.*, 1979). The use of seston may be an important approach for the analysis of feeding activity in the natural habitat.

The present work has shown how much biodeposit may be produced by mussels on rocky shores and how certain factors affect the rate of biodeposit production. These biodeposits may play an important rôle in conditioning coastal waters and the sea bed environment. It is speculated that there may be some differences in the effect of biodeposit between calm water and wave beaten environments. The movement of these large quantities of biodeposit in the coastal water and their effect on the sea bed requires further investigation.

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PODIAL SENSORY RECEPTORS AND THE INDUCTION OF METAMORPHOSIS IN ECHINOIDS

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Abstract: Larvae of *Strongylocentrotus droebachiensis* (Müller), *Lytechinus pictus* (Verrill), and *Lytechinus variegatus* (Leske) which are competent to metamorphose display what appears to be substratum-testing behavior prior to metamorphosis. Larvae cease swimming, partially evert the adult rudiment, and walk about examining the substratum with their five primary podia. Larvae either metamorphose or withdraw their podia and resume swimming to settle again elsewhere. Scanning and transmission electron microscopic examinations of the primary podia revealed sensory receptor cells on the rim and on a conical projection at the center of the podial sucker. Each sensory cell has a single short cilium on its apical surface and an axonal process at its base which contributes to the basiepithelial nerve plexus. Mature adults of the same species also have comparable sensory structures on their tube feet suckers. It is suggested that the sensory receptors on the primary podia of settling larvae, although they are not specialized larval structures, may be involved in the perception of tactile stimuli which have been previously demonstrated to be involved in the induction of metamorphosis.

INTRODUCTION

The distribution of benthic marine organisms is determined in part by the preferential settlement of planktonic larvae in suitable adult habitats (Crisp, 1974; Scheltema, 1974; Chia & Rice, 1978). For some animals it has been demonstrated that the morphological and physiological changes of metamorphosis can be induced by "factors" from preferred adult habitats (Meadows & Campbell, 1972; Scheltema, 1974; Hadfield, 1978). Such is probably the case for many species of echinoids. Strathmann (1978) notes that there are seven species of echinoids which preferentially settle in the presence of substrata associated with adult habitats. Cameron & Hinegardner (1974) have demonstrated for two echinoids that metamorphosis is induced by a non-particulate organic chemical cue and the contact of the larva with a surface. There have been several recent reports of the developmental processes that comprise metamorphosis in echinoids (Cameron & Hinegardner, 1978; Chia & Burke, 1978; Burke, 1980), but very little is known about initiation or control of these processes.

Reports of the settlement and metamorphosis of regular echinoids have described a characteristic form of settlement behavior in which larvae examine the substratum with the five primary podia of the adult rudiment. MacBride (1903), Cameron & Hinegardner (1974), and Strathmann (1978) have suggested that the sucker tips of the primary podia are the location of sensory organs that are responsible for the perception of the substratum-associated cues to metamorphosis. This study was

undertaken to determine if there are recognizable sensory structures on the tips of the primary podia, and if the primary podia are specialized as larval settlement organs.

Materials and Methods

Embryos and larvae were cultured using the standard procedures outlined by Strathmann (1968) and Hinegardner (1969). Adult *Lytechinus pictus* (Verrill) were obtained from Pacific Biomarine Supply, Venice, California, U.S.A.; adult *Strongylocentrotus droebachiensis* (Müller) were collected intertidally on San Juan Island, Washington, U.S.A.; and adult *Lytechinus variegatus* (Leske) were collected from the Indian River near St. Lucie Inlet, Florida, U.S.A. Photographs of live larvae were made using a Nikon inverted microscope and a Zeiss Tessovar photomacrographic system.

Whole larvae and tube feet from adults were prepared for transmission electron microscopy (TEM) by initially fixing in a solution containing 2.5% glutaraldehyde, 0.2 M phosphate buffer (Millonig, 1961), and 0.14 M sodium chloride (Cloney & Florey, 1968). Specimens were post-fixed for 1 h at room temperature in 2% osmium tetroxide in 1.25% sodium bicarbonate (Cloney & Florey, 1968). Dehydration was by acetone exchange and specimens were infiltrated and embedded in Epon (Luft, 1961). Sections were stained with 50% ethanol saturated with uranyl acetate and lead hydroxide chelated with sodium citrate (Reynolds, 1963). Observations and micrographs were made using a Zeiss EM 9S-2 and a Hitachi HU-12 electron microscope.

Specimens were prepared for scanning electron microscopy (SEM) by fixation and post-fixation as described above for TEM. After post-fixation, specimens were rinsed in distilled water and put in a saturated, aqueous solution of thiocarbohydrazide for 15 min, after which they were rinsed and put in 2% osmium tetroxide for 13 min. After a final rinse in distilled water, specimens were dehydrated using alcohol exchange, critical point dried (CO_2), and coated with evaporated carbon and gold-palladium. Specimens were viewed and photographed with an AMR-1000 scanning electron microscope.

Results

SETTLING BEHAVIOR

In larvae competent to metamorphose, the adult rudiment occupies the entire left half of the larval body for all three species studied (Fig. 1). Probably owing to the increased mass of the rudiment, competent larvae were less able to swim at the surface of the water and tended to be found on or near the bottoms of the cul-

ture dishes. The spines and podia of the rudiments of competent larvae were able to move within the vestibule and extend through its dilated opening (Fig. 1). Larvae were able to use the podia to attach themselves to the substratum and walk (Fig. 2).

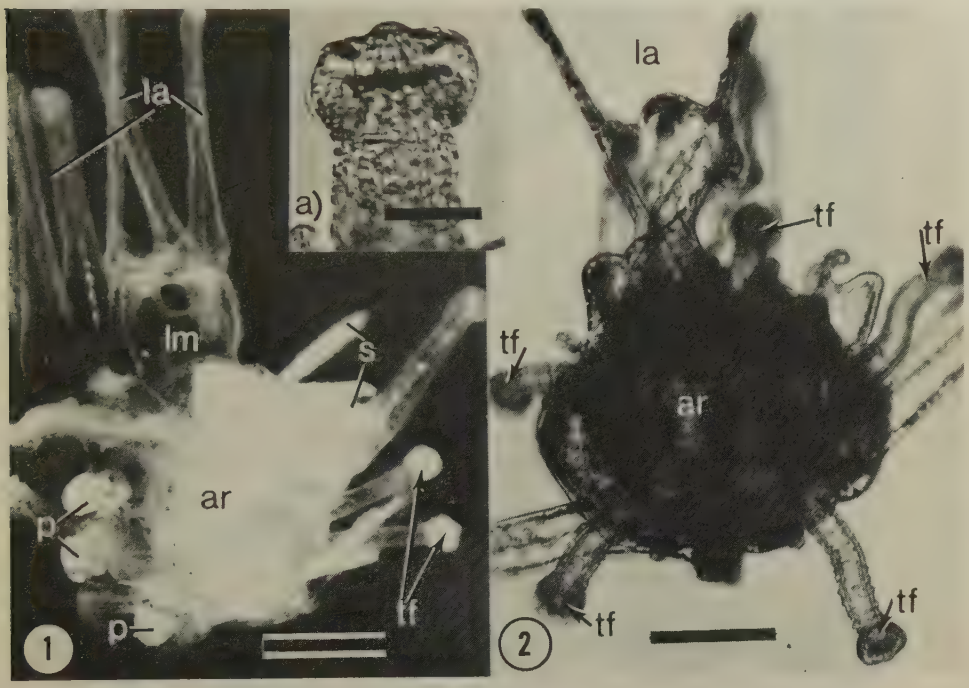

Fig. 1. Pluteus larva of *Lytechinus variegatus* which is competent to metamorphose: note the primary podia (tf) extending from the adult rudiment; bar 100 μm; a, the sucker tip of a primary podium of a competent *L. variegatus* larva; bar 25 μm.

Fig. 2. A competent larva of *Lytechinus pictus* which has settled and is using its primary podia to attach itself to the substratum (a glass dish): the larva was photographed from beneath with an inverted microscope; bar 100 μm; ar, adult rudiment; la, larval arms; lm, larval mouth; p, pedicellariae; s, adult spines; tf, primary podia.

If a suitable substratum was provided for them (rocks and shells from adult habitat) larvae usually underwent metamorphosis. However, if larvae were kept in clean glass bowls, they seldom metamorphosed. Larvae in glass bowls spent prolonged periods (15 min to several days) walking on the bottom. Attached larvae were frequently observed to retract the spines and podia of the rudiment into the vestibule and resume swimming.

STRUCTURE OF THE SUCKERS OF THE PRIMARY PODIA

Competent larvae of all three species have five primary podia evenly spaced around the perimeter of the adult rudiment (Fig. 2). The podia are capable of flexion,

contraction, and extension up to 0.5 mm. Podia are 15–35 μm in diameter, and have a disc-shaped sucker at the distal tip (Fig. 1a). The sucker is 40–45 μm in diameter with an elevated rim and a centrally located cone which projects above the rim (Fig. 1a).

The podial sucker is comprised of two tissue layers separated by a connective tissue layer (Fig. 3). The inner layer is part of the water vascular system and consists of a tapered cylinder with muscular walls which extends into the central cone of the sucker. The tip of this inner cylinder is usually filled with mesenchymal cells.

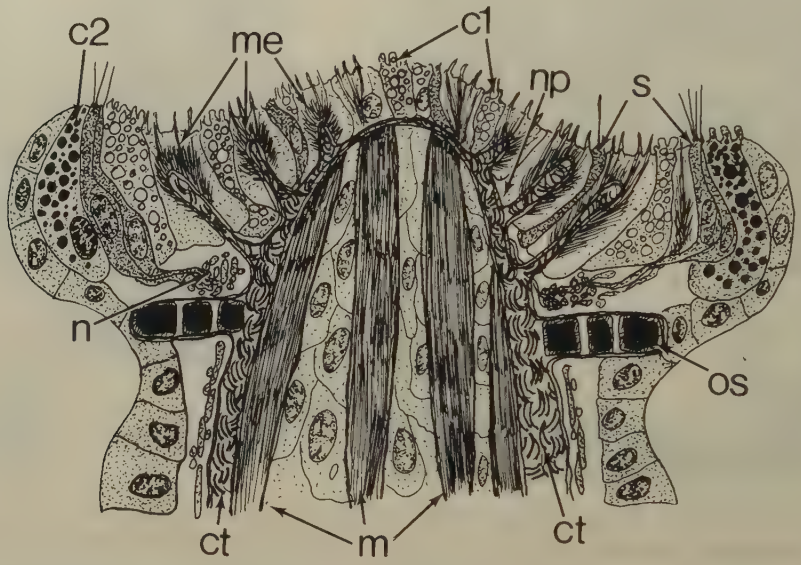

Fig. 3. A schematic drawing of a sagittal section through a primary podium of a competent larva of *Strongylocentrotus droebachiensis*: c1, Type I collocyte; c2, Type II collocyte; ct, connective tissue; m, muscle layer of water vascular system; me, myoepithelium; n, nerve ring; np, basiepithelial nerve plexus; os, ossicle; s, sensory cells.

The connective tissue layer is made up of collagen-like fibers and varies in thickness from 0.5 3 μm. At the base of the sucker, within the connective tissue layer there is a discoid, calcareous ossicle. On the sides of the central cone, the connective tissue layer extends finger-like projections into the overlying epithelium of the sucker. The sucker epithelium is a simple, pseudostratified epithelium predominantly made up of myoepithelial and secretory cells. The epithelium covering the entire podia is underlain with a basiepithelial nerve plexus. Also, at the base of the sucker is located a nerve ring associated with the distal surface of the ossicle.

The myoepithelial cells are 4–5 μm wide, up to $\approx$10 μm long and appear to surround the finger-like projections of connective tissue (Fig. 4). Arrays of filaments

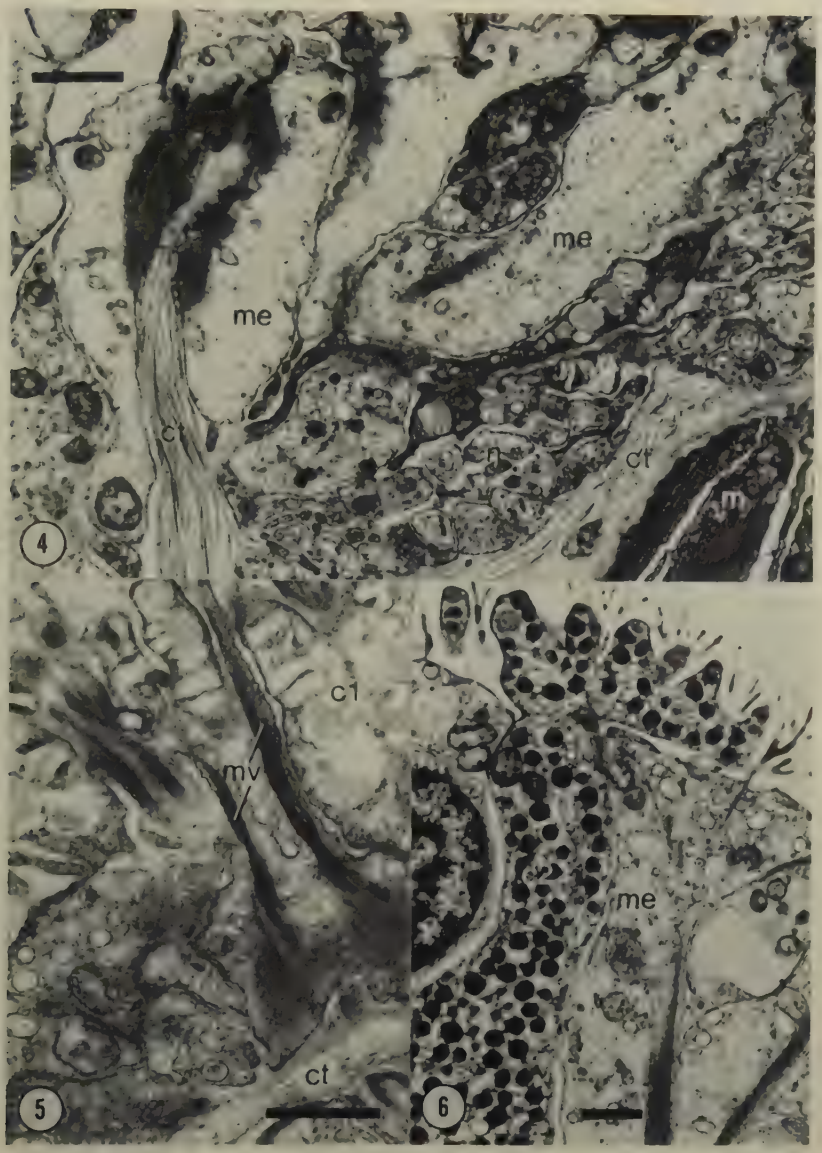

Fig. 4. Transmission electronmicrograph of the sucker epithelium of a primary podium of a competent larva of *Strongylocentrotus droebachiensis*: bar 1 μm; ct, connective tissue; m, inner muscle layer; me, myoepithelial cells; n, basiepithelial nerve plexus.

Fig. 5. Transmission electronmicrograph of a portion of the myoepithelium of the sucker of a competent larva of *Strongylocentrotus droebachiensis*: bar 1 μm; cl, Type I collocyte; ct, connective tissue; mv, microvilli of a myoepithelial cell.

Fig. 6. Transmission electronmicrograph of a region on the rim of the sucker from the primary podium of a competent larva of *Lytechinus pictus*: bar 1 μm; c2, Type II collocyte; me, myoepithelial cell.

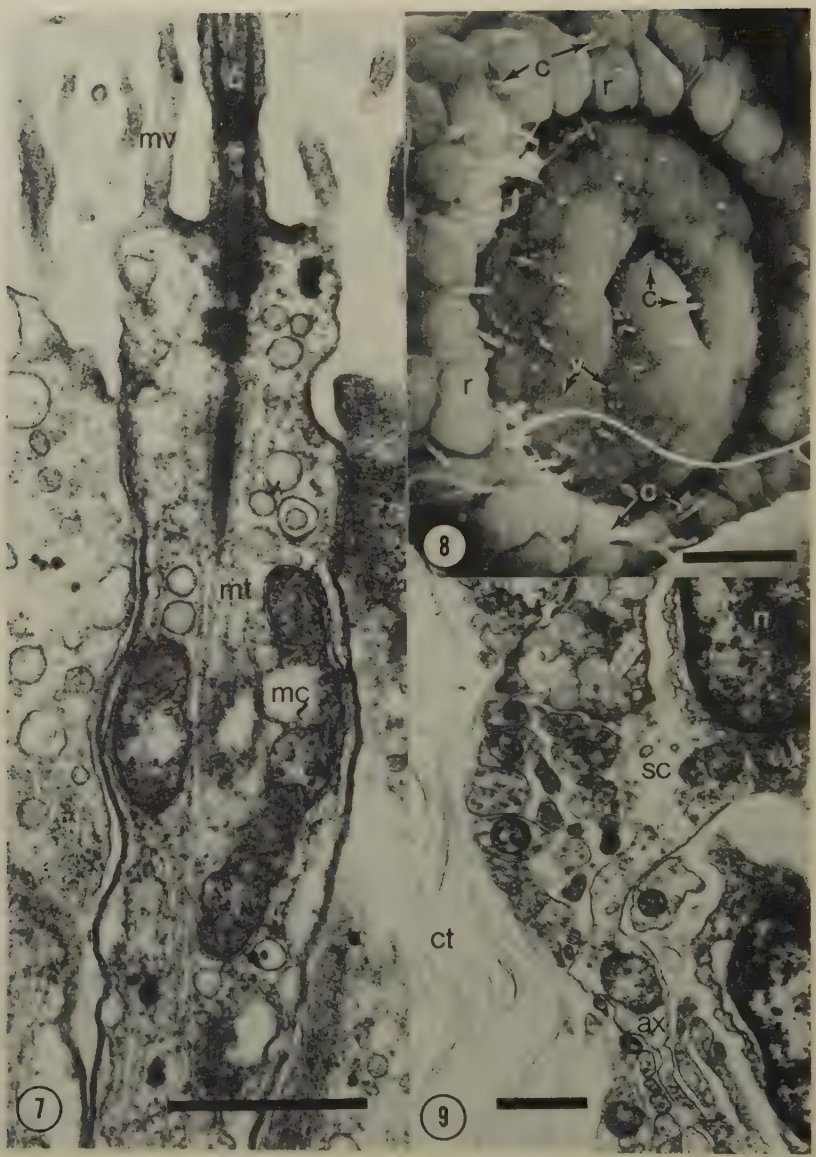

Fig. 7. Transmission electronmicrograph of the apical portion of a sensory cell from the sucker epithelium of a competent *Strongylocentrotus droebachiensis* larva: bar 1 μm; c, cilium; mc, mitochondria; mt, microtubules; mv, microvilli.

Fig. 8. Scanning electronmicrograph of the sucker of the primary podium of a *Strongylocentrotus droebachiensis* larva: bar 10 μm; c, cilia; r, rim; v, vesicles.

Fig. 9. Transmission electronmicrograph of a region of the basiepithelial nerve plexus subjacent to the sucker epithelium of a primary podium of a larva of *Strongylocentrotus droebachiensis*: note the basal axonal extension of a sensory cell contributing to the nerve plexus; bar 1 μm; ax, axon; ct, connective tissue; n, nucleus; sc, sensory cell.

oriented along the major axis of the cell extend between the connective tissue core and the apical surface of the cell. Microvilli, 1–2 μm long, on the apical surface of the cells each contain a core of filaments which are an extension of the filaments of the main portion of the cell (Fig. 5). Otherwise, the cytoplasm of the myoepithelial cells is relatively vacant containing only scattered mitochondria, microtubules, and a few vesicles.

The secretory cells of the sucker epithelium appear to be of two morphologically distinct types. Type I collocytes occur over the entire surface of the sucker and probably correspond to the secretory cells described from the tube feet suckers of holothuroids (Harrison, 1968) and asteroids (Harrison & Philpott, 1966; Souza Santos, 1966). The cells are 4–5 μm wide, 10 μm long, and are interspersed with the myoepithelial cells (Fig. 5). Type I collocytes have characteristic secretory vesicles which contain condensed flocculent material in a central band and around the periphery of the vesicle (Fig. 5). The apical surface of the Type I collocytes is comprised of numerous irregular projections packed with secretory vesicles. The Type II collocytes are restricted in their distribution to the rim of the sucker and contain numerous electron dense vesicles. The cells are 3 μm wide and up to 15 μm long (Fig. 6). The apical surface of the Type II collocytes is elaborated into projections similar to the surface projections of the Type I collocytes.

Bipolar sensory cells occur over the entire sucker epithelium. The sensory cells are clumped into groups of three to five cells on the rim of the sucker, while they occur singly over the central regions (Fig. 8). Each cell is up to 1 μm wide and 15 or 20 μm long (Fig. 7). The apical surface has a single cilium which is only 3–4 μm long, and is surrounded at its base by a collar of 0.5-μm long microvilli. In favorable sections, the basal ends of the sensory cells can be seen to taper to axonal processes, 0.1–0.2 μm in diameter, which contribute to the basiepithelial nerve plexus (Fig. 9). Mitochondria, microtubules, and 0.1-μm clear vesicles are the predominant organelles of the sensory cells.

STRUCTURE OF THE TUBE FEET SUCKERS OF ADULTS

For the three species studied, the form of adult tube feet is essentially the same as the form of the primary podia of competent larvae. However, adult tube feet are much larger; they are able to extend to ≈8 cm and are ≈0.5 mm in diameter. The histological organization of adult tube feet is the same as that of the primary podia with the notable difference that the tissues are much thicker. The epithelium of the sucker is ≈200 μm thick, and comprised of stratified, columnar cells (Fig. 10). The sucker epithelium is made up of the same cell types as the podial sucker epithelium, and the cells have essentially the same ultrastructural characteristics as those of the primary podia described above.

Sensory cells are dispersed over the entire surface of the adult tube feet suckers. The sensory cells on the rim of the sucker are clumped into groups of about 50 cells

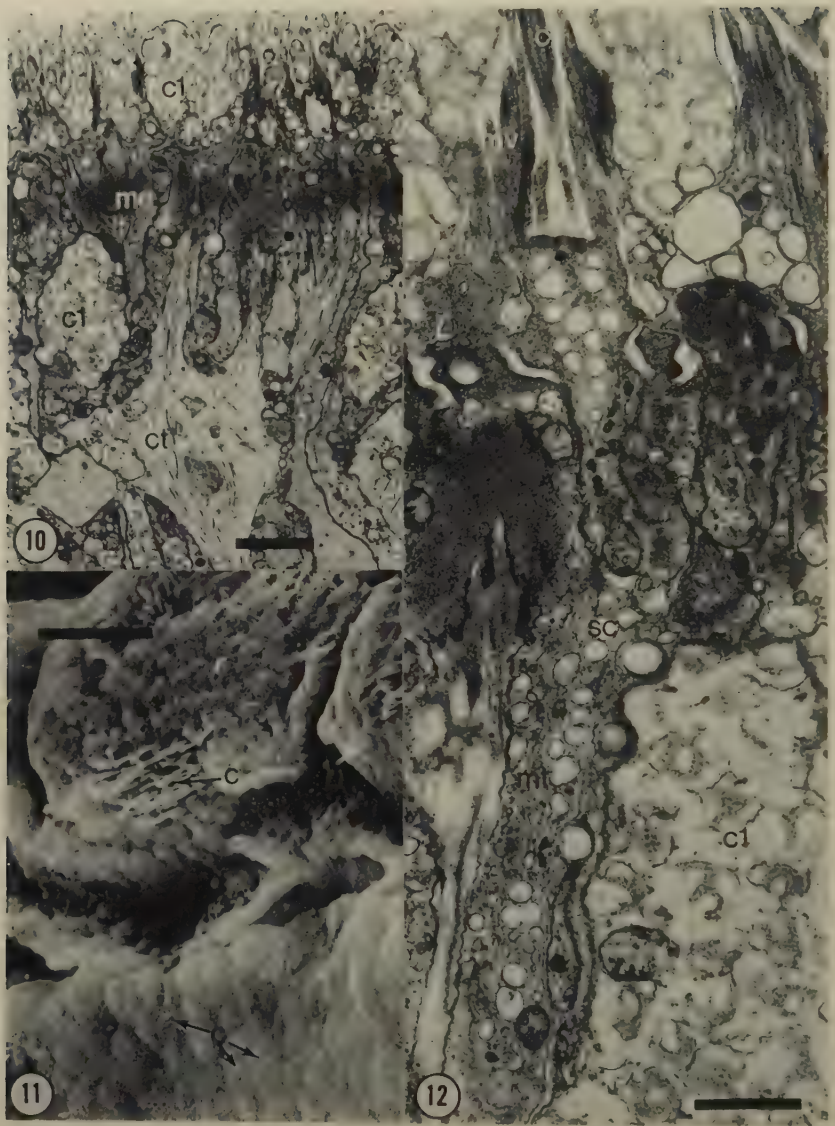

Fig. 10. Transmission electronmicrograph of the sucker epithelium of the tube foot of an adult *Strongylocentrotus droebachiensis*: bar 3 μm; cl, type I collocyte; ct, connective tissue; me, myoepithelium.
Fig. 11. Scanning electronmicrograph of a portion of a sucker, near the rim, of a tube foot of an adult *Strongylocentrotus droebachiensis*: bar 5 μm; c, cilia; r, rim.
Fig. 12. Transmission electronmicrograph of the apical portion of a sensory cell from the sucker epithelium of a tube foot of an adult *Strongylocentrotus droebachiensis*: bar 0.5 μm; c, cilium; cl, Type I collocyte; mt, microtubules; mv, microvilli; sc, sensory cell.

and the sensory cells in the central region occur singly (Fig. 11). Each sensory cell bears a single short cilium on its apical surface, while a collar of microvilli surrounds it at its base (Fig. 12). The basal end of the sensory cells extends as a thin axonal process into the basiepithelial nerve plexus. The cytoplasm of the sensory cells is characterized by microtubules, mitochondria, and clear vesicles.

Discussion

The sensory cells described here are similar to those described from other phyla (Bullock & Horridge, 1965). Specifically, the apical cilium and the basal axonal process are characteristics shared by many superficial sensory organs (Laverack, 1968, 1974; Bedini *et al.*, 1973; Bonar, 1978). The functions most commonly ascribed to superficial sensory organs of this type are mechanoreception and chemoreception (Laverack, 1968, 1974). Horridge (1965) has reviewed the modifications described from different sensory organs and suggested that it is not possible to determine the precise function of sensory organs from their structure. Similarly the sensory structures described here probably serve as receptors for some external stimuli, but it is not possible to state unequivocally the nature of those stimuli from structural evidence alone.

The ability of the larvae to come in contact with a benthic habitat, then retract their tube feet and resume swimming, suggests that the larvae are actually searching for an appropriate place to settle. Cameron & Hinegardner (1974) concluded that at least two conditions had to be met to induce metamorphosis in *Lytechinus pictus* and *Arbacia punctulata*: (1) the presence of a soluble organic chemical cue, and (2) contact of the larva with a surface. Cameron & Hinegardner (1974) found larvae in sea water containing the chemical cue require a prolonged (several minutes) contact of the podia with a solid surface for metamorphosis to proceed. In 80% of their tests, larvae held so that the podia were not allowed to touch a surface, would not metamorphose. Releasing the larvae or allowing them to contact a surface permitted metamorphosis to proceed. It is difficult to determine the location of sense organs which perceive the diffuse chemical cue, but it seems likely that the sensory structures on the tips of the primary podia may be employed at least in tactile perception of the substratum.

The observations of Cameron & Hinegardner (1974) apply to *Lytechinus pictus* and *Arbacia punctulata* and it is not certain that all echinoids have a similar set of cues to metamorphosis. Indeed, *Mellita quinquesperforata* and *Dendraster excentricus* metamorphose without the preliminary settling behavior reported here (Caldwell, 1972; Burke, unpublished observations). Yet both of these species appear to be induced to metamorphose by some substratum-associated factor from the adult environment (Caldwell, 1972; Highsmith, 1977). Neither *Lytechinus variegatus* nor *Strongylocentrotus droebachiensis* have been demonstrated to require anything more

than a surface for metamorphosis. Undoubtedly, there are several types of cue for metamorphosis in echinoids. One characteristic shared by many of them is that settlement precedes or is coincidental with metamorphosis (Hyman, 1955; Strathmann, 1978). Conceivably, echinoid larvae share some means of sensing the immediate presence of a surface; some species may rely on chemical cues, others evidently employ a tactile sense and some may require both forms of stimuli.

The sensory cells described on the podial suckers are not exclusively larval structures. There are some differences in the size and number of sensory cells on the podia of adults as compared to those of the larva, but the distribution appears to be the same for both larva and adult. The sensory cells may function primarily in the control and coordination of the sucker itself. The myoepithelium, the muscular wall of the inner layer, and the two types of collocytes may all be under nervous control which originates with sensory input from the sensory receptor cells of the podial epithelium. The control of podial function is not at all clear from the structural data available (Florey & Cahill, 1977; Cobb & Raymond, 1979). However, the observation of sensory structures on the sucker may infer that they are autonomously controlled in a manner similar to that suggested for pedicellariae (Laverack, 1968).

Barker (1978) described the structure of the organs of attachment of brachiolarian larvae of two species of asteroids and noted ciliary sense cells that he suggested are used in the recognition of substrata suitable for settlement of the larva. Brachiolarian arms are specialized larval structures that appear to function solely during the settlement of the larva (reviewed by Strathmann, 1978) and so their role in identification of substratum-associated cues seems apparent. Echinoid larvae lack structures homologous to brachioloarian arms, but the primary podia do play an analogous role during settlement and metamorphosis. The receptors on the podia probably do not function only during settlement, but appear to be more generalized sensory elements that may control podial suckers throughout the life of the urchin. Tactile stimulation of the podial nervous system when combined with other cues associated with suitable adult habitat may serve as the initial steps in the induction of metamorphosis in echinoid larvae.

Acknowledgements

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THE EFFECTS OF SALINITY, AND CALCIUM AND POTASSIUM VARIATIONS ON THE GROWTH OF TWO ESTUARINE RED ALGAE

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Abstract: The effects of varying salinity and calcium and potassium concentrations on the growth of two species of estuarine Rhodophyta, *Bostrychia radicans* Montagne and *Caloglossa leprieurii* (Montagne) J. Agardh, were examined in unialgal culture. Inocula of settled tetraspores on glass coverslips were incubated in six concentrations of potassium (0, 100, 200, 300, 400 and 500 mg/l) at five salinities (0, 5, 15, 25 and 35‰) in the presence or absence of calcium. Growth responses of each alga were determined from the average cell number of 75 tetrasporelings after 4 days in Ott's synthetic sea-water medium. The concentrations of dissolved potassium and calcium in sea water along a salinity gradient in an estuary were determined with atomic absorption spectrophotometry. Four-day-old tetrasporelings of *Bostrychia* and *Caloglossa* demonstrated growth over wide ranges of potassium concentrations with growth maxima at 400–500 and 200–400 mg/l, at the optimal salinities for growth of 25 and 15‰, respectively. These studies indicate also that the presence of calcium is essential for appreciable growth of both species at each salinity and the effects of variations in potassium are dependent upon the presence of calcium. The abundance of both species in the Mullica River estuary, New Jersey, appears to be a response to a total osmotic effect along the salinity gradient because sufficient levels of the major cations are present. However, the upper limit of both species towards the head of the Mullica River estuary may be determined by combinations of low salinity and low levels of dissolved calcium and potassium rather than by low salinity alone.

INTRODUCTION

Previous investigations on the effect of salinity on the distribution of benthic marine algae indicate that it is a major factor limiting species composition in an estuary (Den Hartog, 1967; Kinne, 1967; Gessner & Schramm, 1971; Khfaji & Norton, 1979). Salinity is primarily controlled by the prevailing ebb and flow of the tides, as well as by the amount of freshwater runoff which usually varies seasonally. Fluctuations in salinity result in changes in density, osmotic pressure, ionic strength, electroconductivity, hydrogen ion concentration, and differences in

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the amount of each salt present in solution (Conover, 1964). Furthermore, the relative ion composition also undergoes changes when the ion composition of the diluting water differs from that of the sea water. Such changes in ion concentrations are most pronounced at salinities of 8‰ and lower (Khlebovich, 1968). Further changes in ion ratios may result from base exchange, or the replacement of calcium in river-borne clays by sodium, potassium, and magnesium (Martin, 1970).

The ionic composition of the surrounding water has been shown to be an important factor in the effect of salinity on organisms (Provasoli, 1958; Guillard, 1962; Lewin & Guillard, 1963; Gessner & Schramm, 1971). Calcium is an ion of special importance in maintaining organisms at otherwise lethal salinities. Hoffman (1959) demonstrated that by increasing the concentration of calcium in the external media, *Delesseria sanguinea* and other red algae could survive otherwise fatal salinities of 10, 5, and 1‰. McIntosh (1978) similarly found that *Bostrychia binderi* photosynthesized for relatively long periods at salinities between 0 and 5‰ in the spring waters from the west coast of Florida because of the high level of calcium.

Physiological mechanisms of ion uptake, ion loss, and ion transport have been reviewed by Gutknecht & Dainty (1968) and MacRobbie (1970, 1974). It is now thought that calcium exerts its primary effect at the membrane level by decreasing membrane permeability (Eppley & Cyrus, 1960; Poovaiah & Leopold, 1976), maintaining membrane selectivity (Epstein, 1972; Nuccitelli & Jaffe, 1976a) and integrity (Schwenke, 1958a; LaHayne & Epstein, 1969; Rains, 1972), as well as by increasing the width of the membrane (Moore & Bracker, 1976). It has also been suggested that calcium may have an effect on stabilizing the cell wall by reacting with major extra-cellular polysaccharides (Eppley & Cyrus, 1960). Potassium, another major cation, primarily serves a number of catalytic roles, as an activator and cofactor of enzymes in respiration and carbohydrate metabolism (Epstein, 1972). It may also be involved in membrane permeability, especially in maintaining turgor pressure regulation in certain marine algae (Zimmerman & Steudle, 1971; Soeder & Stengel, 1974; Nuccitelli & Jaffe, 1976b).

There is a paucity of information available on the effects of the simultaneous variations of salinity, and calcium and potassium on the growth of benthic marine algae. Most work has been oriented towards a physiological point of view and little attention has been focussed on correlating these results with the autecology of marine algae. Norton & South (1969) and Nygren (1975) stressed the need for experiments in culture to investigate the interactions between salinity and the ionic composition of the ambient medium on the growth and development of seaweeds. This communication reports an attempt to determine the effects of salinity and calcium and potassium variations on the growth of *Bostrychia radicans* Montagne and *Caloglossa leprieurii* (Montagne) J. Agardh and to ascertain the relative importance of each factor in controlling the horizontal distribution of these algae in an estuary.

Materials and Methods

The Mullica River estuary is ≈17 km E.N.E. of Atlantic City, New Jersey, U.S.A. (Fig. 1). The drainage basin includes about 15 km^2 of low outer coastal plain, a large portion of which drains the relatively unpolluted New Jersey Pine Barrens.

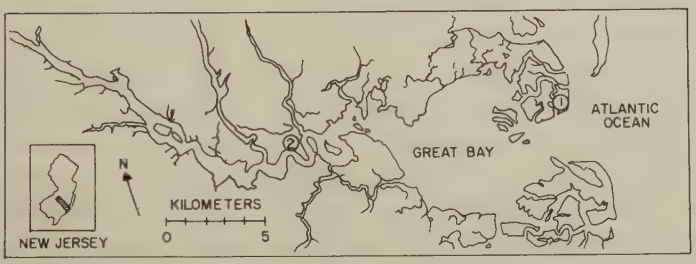

Fig. 1. Map of the Mullica River estuary: Station 1, Marine Biology Station; Station 2, Parkway Bridge.

The unialgal cultures of benthic marine algae used in the study were isolated from this estuary. The habitats, locations, and dates of collection of the two isolates are as follows: *Bostrychia radicans*, upper eulittoral zone, wooden pilings, Marine Biology Station, 17 July, 1973; and *Caloglossa leprieurii*, mideulittoral zone, wooden pilings, Parkway Bridge, 13 July, 1973. The salinity regime of the Marine Biology Station, near the mouth of the estuary, varied from 22.56 to 30.61‰ and that of the Parkway Bridge location, near the head of the estuary, varied from 4.76 to 18.50‰ measured over a period of several months. Cystocarpic fronds of each alga were placed in 60 × 15 mm Pyrex Petri dishes containing sterile Von Stosch's enriched sea-water medium, as cited by Ott (1966), and left overnight. The following morning the released carpospores were isolated into unialgal culture. The stock cultures of *Bostrychia* were maintained in Von Stosch's enriched sea-water medium at 25‰ and those of *Caloglossa* at 23 ± 1 °C, on a 16 : 8 DL with cool-white fluorescent illumination at 1976 ± 161 lx. The integrity of the original genotypes was maintained in culture from cuttings of the apical branches of the tetrasporophytes.

A series of experiments was designed to investigate the effects of the simultaneous variation of salinity and calcium and potassium on the growth of tetrasporelings in a synthetic sea-water medium (Ott, 1965). Growth responses of each alga were determined from the average cell number of 75 tetrasporelings according to the procedure described by Edwards (1977). The cell numbers represent the number of cells in the axial row and the longest rhizoid because the tetrasporelings became pluriseriate. Inocula of settled tetraspores on glass coverslips were incubated at five salinities (0, 5, 15, 25, 35‰), and at six concentrations of potassium (0, 100, 200, 300, 400, 500 mg/l) in the presence or absence of calcium. The cultures in each experimental regime were grown in 100 × 15 mm Pyrex Petri dishes which were

washed in 10% nitric acid. To preclude any limitations of CO_2 for photosynthesis, the concentration of carbonate buffers in the artificial sea-water medium was the same in all salinity regimes (Tseng & Sweeny, 1946; Ogata & Matsui, 1965; Hammer, 1968). The pH of all media was adjusted to 8.0 when necessary by the addition of HCl or NaOH. The cation levels of the synthetic sea-water medium were made up to approximate the composition of sea water at the Marine Biology Station at mean high tide. Equimolar equivalents of rubidium and strontium were substituted for potassium and calcium, respectively, to maintain the osmotic potentials of the solutions. A freshwater basal medium with a salinity approaching 0‰ was prepared by the addition of 0.03 g/l NaCl, 0.03 g/l $CaCl_2$, and 0.03 g/l $MgSO_4 \cdot 7H_2O$ to the modified Ott's synthetic sea-water medium lacking the salts NaCl, $MgSO_4$, $MgCl_2 \cdot 6H_2O$ and $CaCl_2 \cdot 2H_2O$. The freshwater basal medium contained 15 mg/l calcium, which was higher than the freshwater runoff from the New Jersey Pine Barrens (≈ 1 mg/l). The effects of increasing levels of calcium upon growth of *Bostrychia* and *Caloglossa* tetrasporelings at optimal salinity levels of 25 and 15‰, respectively, were also investigated. The concentrations of calcium in the culture media were 0, 100, 200, 250, and 300 mg/l. The final potassium, calcium, sodium, and magnesium concentrations of all solutions were verified by a Perkin-Elmer 305B double beam atomic absorption spectrophotometer, including media free of calcium and potassium. Reagent grade chemicals were used to make up the medium. The light intensity and temperature were held constant at 2252 ± 269 lx and 26 ± 1 °C, respectively, in a Sherer Dual Jet Incubator, Model RT 26 B-SE (Marshall, Michigan).

Data were analyzed by two- and three-way ANOVAs and Tukey's multiple comparison on the means (= Tukey's w-procedure, Miller, 1966) and tested for significant differences at the 5% level. The interaction effects between calcium and salinity, and those between potassium and salinity were summarily analyzed utilizing Scheffé's method (Scheffé, 1967).

Results

Variations in the ionic composition of the sea water during a tidal cycle at the head and mouth of the Mullica River estuary are presented in Table I.

Tetrasporelings of *Bostrychia* showed a broad growth response to variations in salinity and calcium and potassium levels (Figs. 2–5). *Bostrychia* was euryhaline, with optimal growth between 15–35‰ S and a significant decline in growth at 5‰ S in the presence of calcium (Fig. 2, $P < 0.05$). Growth was dependent upon the presence of calcium at all salinities (Figs. 2, 3, Table II). The effect of calcium was particularly pronounced at 15‰ S and least evident at 5‰ S (Table II). Correspondingly, calcium was also beneficial to growth at all the potassium levels (Figs. 2, 3). Furthermore, there was a significant interaction in that the effect of calcium

TABLE I

Variations in the salinity, pH and major dissolved cations during a tidal cycle at the head and mouth of the Mullica River estuary on 25 May, 1976: water samples were taken at approximately hourly intervals.

	Tidal level	Salinity (‰)	pH	Cations (mg/l)			
				K^+	Ca^{2+}	Na^+	Mg^{2+}
Head of estuary (Parkway Bridge)	Low	9.0	7.3	125	115	3530	290
		10.0	7.3	135	125	3770	310
		11.0	7.4	155	126	3770	350
		13.0	7.5	165	135	3830	390
		14.0	7.5	180	143	4000	400
		17.0	7.7	195	175	5030	470
	High	18.5	7.7	205	183	6170	540
Mouth of estuary (Marine Biology Station)	Low	28.0	8.0	315	244	7800	710
		29.0	8.0	320	254	8130	750
		29.0	8.0	325	255	8230	760
		29.0	8.1	335	261	8600	780
		29.0	8.1	340	265	9030	780
		30.0	8.1	380	272	9100	810
	High	30.0	8.1	390	287	9700	910

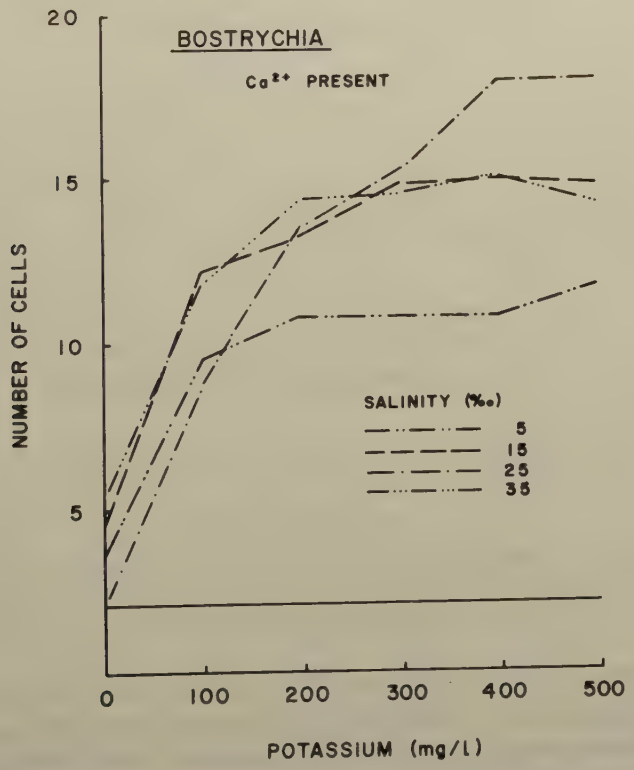

Fig. 2. The growth of *Bostrychia* cultured in varying salinity and potassium concentrations in the presence of calcium (average number of cells per 75 tetrasporelings after 4 days): horizontal line in the lower portion of the graph represents the average number of cells of the inoculum (Tukey's $w = 1.22$).

TABLE II

Statistical summaries of the tetrasporeling growth of *Bostrychia radicans* as a function of variations in salinity and potassium and calcium treatments: analyses by Tukey's multiple comparison on the means of Scheffé's method on the interactions at the 5% level; any values underscored by the same line are not significantly different; the magnitude of the growth responses are ranked from smallest to largest, left to right.

Treatments	Multiple comparisons					
Potassium (Tukey's *w*-procedure) (mg/l)	0	100	200	300	400	500
Salinity (Tukey's *w*-procedure (‰))	5	15	35	25		
Calcium × salinity (Scheffé's method)	5	25	35	15		
Calcium (Scheffé's method)	0	100	200	300	500	400

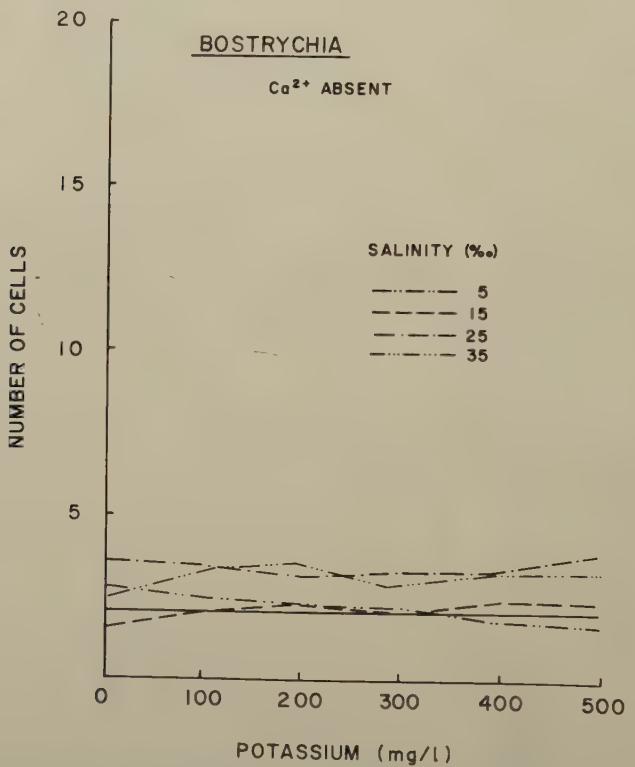

Fig. 3. The growth of *Bostrychia* cultured in varying salinity and potassium concentrations in the absence of calcium (average number of cells per 75 tetrasporelings after 4 days): horizontal line in the lower portion of the graph represents the average number of cells of the inoculum ($w = 1.22$).

was strongest at the levels of potassium of at least 300 mg/l (Table II). Very little, if any growth occurred at 0‰ *S*; however, in the presence of calcium, tetrasporelings grown in 400 and 500 mg/l potassium did have significantly higher growth than those grown in 300 mg/l and less (Fig. 4). Even though there was a 2 to 4 mg/l

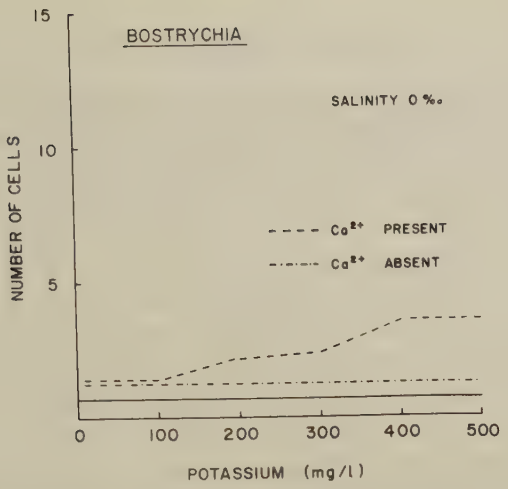

Fig. 4. The growth of *Bostrychia* cultured in varying potassium concentrations in the presence or absence of calcium at 0‰ (average number of cells per 75 tetrasporelings after 4 days): horizontal line in the lower portion of the graph represents the average number of cells of the inoculum ($w = 1.49$).

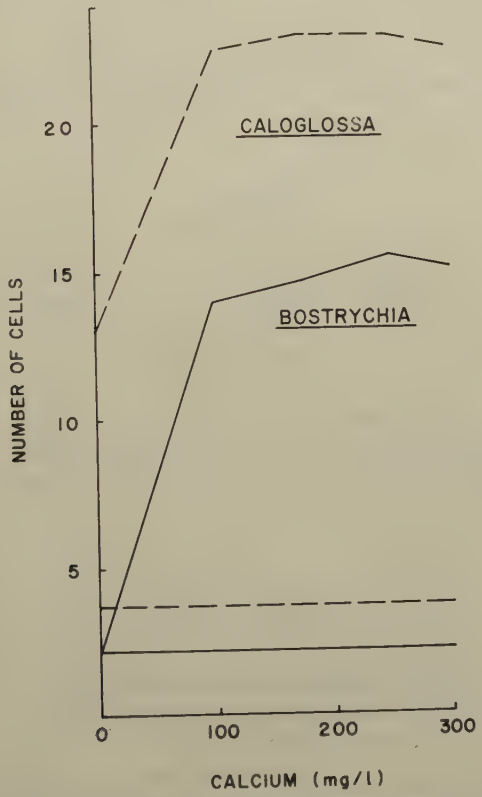

Fig. 5. The growth of *Bostrychia* ($w = 0.82$) and *Caloglossa* ($w = 1.03$) in varying concentrations of calcium at 25 and 15‰, respectively (average number of cells per 75 tetrasporelings after 4 days): horizontal lines in the lower portion of the graph represent the average number of cells of the inoculum of each alga.

calcium contamination level in the medium lacking calcium, as determined by atomic absorption spectrophotometry, there was no significant growth in the treatment medium. Maximum growth as a function of potassium occurred from 400 to 500 mg/l (Fig. 2, Table II). Growth was significantly enhanced at 25‰ S at 400 to 500 mg/l potassium, providing that calcium was present (Fig. 2). Growth at an optimal salinity of 25‰ was saturated at 100 mg/l calcium (Fig. 5).

Tetrasporelings of *Caloglossa* tetrasporophytes also showed a broad response to variations in salinity and potassium and calcium levels (Figs. 5 8). *Caloglossa* was euryhaline, with optimal growth at 15‰ S ($P < 0.05$) and hardly any growth at 35‰ S (Fig. 6). Even though growth was dependent upon the presence of calcium at all salinities (Figs. 6, 7), its presence was most important at 15‰ and least at 35‰. A particularly pronounced effect was evident at 0‰ S (Fig. 8, $P < 0.05$). However, it should be pointed out that a 2 to 4 mg/l calcium contamination level was found in media lacking calcium. Maximum growth in varying levels of potassium occurred at 200 to 400 mg/l ($P < 0.05$), although good growth occurred from 100 to 500 mg/l (Fig. 6). Growth was significantly enhanced at 15‰ ($P < 0.05$) at potassium levels of 200 to 500 mg/l in the presence of calcium (Fig. 6). An interaction at a salinity of 25‰ and 100 mg/l potassium significantly increased growth ($P < 0.05$). There was a statistically significant increase in growth in the

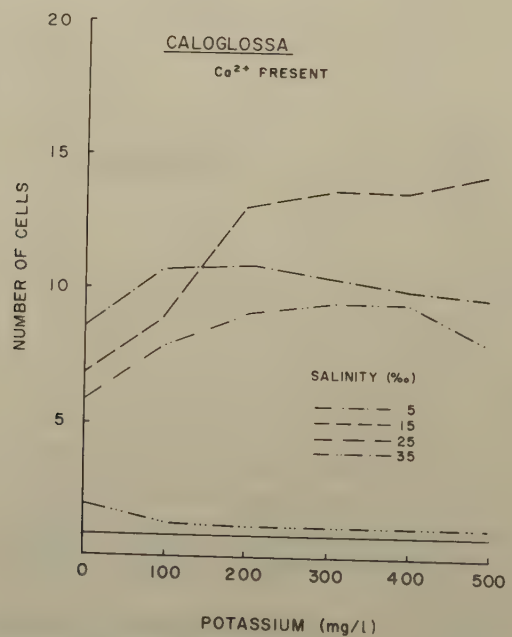

Fig. 6. The growth of *Caloglossa* cultured in varying salinity and potassium concentrations in the presence of calcium (average number of cells per 75 tetrasporelings after 4 days): horizontal line in the lower portion of the graph represents the average number of cells of the inoculum ($w = 0.80$).

presence of calcium at all potassium levels (Figs. 6, 7). This improvement in growth was most evident at 200 to 500 mg/l potassium (Table III). In the freshwater basal medium, growth was significantly stimulated by the presence of calcium and at

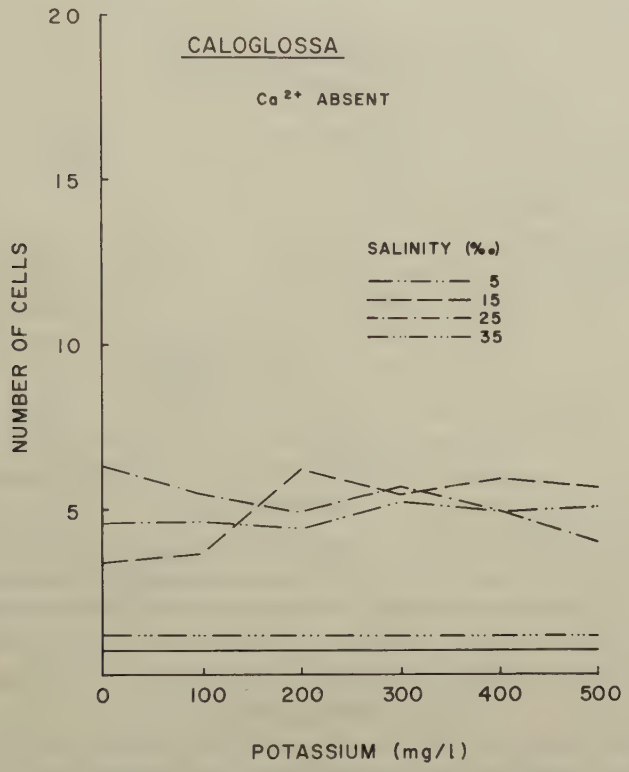

Fig. 7. The growth of *Caloglossa* cultured in varying salinity and potassium concentrations in the absence of calcium (average number of cells per 75 tetrasporelings after 4 days): horizontal line in the lower portion of the graph represents the average number of cells of the inoculum ($w = 0.80$).

TABLE III

Statistical summaries of the tetrasporeling growth of *Caloglossa leprieurii* to variations in salinity and potassium and calcium treatments: analyses by Tukey's multiple comparison on the means and Scheffé's method on the interactions at the 5% level; any values underscored by the same line are not significantly different; the magnitude of the growth responses are ranked from the smallest to largest, left to right.

Treatments	Multiple comparisons					
Potassium (Tukey's *w*-procedure) (mg/l)	0	100	500	200	400	300
Salinity (Tukey's *w*-procedure (‰))	35	5	25	15		
Calcium × salinity (Scheffé's method)	35	5	25	15		
Calcium × potassium (Scheffé's method)	0	100	300	400	200	500

increased levels of potassium (Fig. 8). The growth of *Caloglossa* at an optimal salinity of 15‰ increased with increasing levels of calcium and was saturated at 100 mg/l calcium (Fig. 5). The increase in growth at 0 mg/l calcium suggests that growth may have been supported by strontium in the calcium-poor medium.

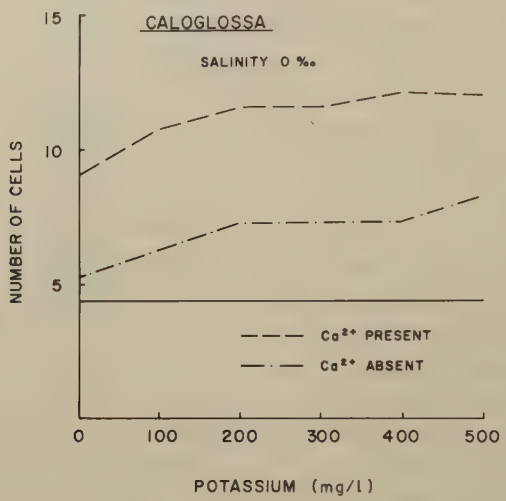

Fig. 8. Growth of *Caloglossa* cultured in varying potassium concentrations in the presence or absence of calcium at 0‰ (average number of cells per 75 tetrasporelings after 4 days): horizontal line in the lower portion of the graph represents the average number of cells of the inoculum ($w = 0.71$).

Discussion

Salinity is one of the major factors which limits the distribution of benthic marine algae in an estuary, but investigators have rarely considered other factors which may be involved in the effects of salinity, such as the ionic composition of the sea water. The ecological importance of tolerance to variations in the ambient ionic composition for *Bostrychia* and *Caloglossa* cannot be overemphasized, since they are constantly being subjected to sea water of varying ionic composition in nature. Variations in ionic composition and salinity during a tidal cycle have been found to be considerable at the two principal study areas. In addition to an approximate doubling of salinity at the mouth relative to that at the head of the estuary, the levels of calcium and potassium increased significantly (Table I). This indicates that algae in the estuary must be able to tolerate wide variations in salinity, and calcium and potassium.

Schwenke (1958b) has emphasized the dependence of cell integrity on calcium and potassium for several species of benthic algae. He observed that the absence of calcium, and to a lesser degree potassium, caused the highest degree of cell damage.

The tolerance limits to the absence of each cation varied with the species, although damage to the plants increased with increasing exposure time. In the present study, growth of tetrasporelings of *Caloglossa* and *Bostrychia* was dependent upon the presence of calcium at all salinities. Little growth occurred in media lacking calcium in salinities from 5 to 35‰. In the freshwater basal medium the growth of *Caloglossa*, and to a lesser extent *Bostrychia*, was significantly stimulated by the presence of calcium. Schwenke (1959) and Zavodnik (1975) have reported that the damage in distilled water is much greater than in media containing calcium. Similar findings have been reported by McIntosh (1978) for *Bostrychia binderi* at low salinities. Plants maintained a higher photosynthetic rate for a longer period of time in spring water rich in calcium than in distilled water alone. He suggests that the increased calcium concentration may exert some effect both on maintaining the membrane integrity and cell wall polysaccharides which probably results in the survival of *B. binderi* at low salinities for longer periods.

At the optimal salinities of 25‰ for *Bostrychia* and 15‰ for *Caloglossa*, growth of tetrasporelings was saturated at 100 mg/l calcium. Since *Caloglossa* did grow in media lacking calcium, the tetrasporelings may have had enough endogenous calcium for limited growth and/or calcium may have been replaced by strontium. However, Schwenke (1958b) reported for *Delesseria sanguinea* that the direct substitution of strontium for calcium at salinities of 10 to 20‰ had no effect upon cell survival up to 14 days. Plants growing in salinities of 1 to 5‰ survived less in the strontium substituted medium. On the other hand, Gilbert & O'Kelley (1964) have found that there may be limited strontium substitution for calcium in the freshwater green alga, *Chlorococcum echinozygotum Starr*. They suggested that strontium may substitute for calcium in unidentified physiochemical requirements of the cell, but that strontium cannot replace calcium as an enzyme activator because the former is too large an ion. Sommerfeld (1976), working with *Goniotrichum*, and Moss *et al.* (1971), working with several freshwater algal species, reported that mitosis and cell division were selectively retarded in a strontium substituted medium. Weiss *et al.* (1976) showed that strontium could not replace calcium in cell division for *Criscosphaera carterae*; however, in low calcium media (a few milligrams per liter) strontium permitted more calcium to enter the well with the result that division occurred. Epstein (1972) reported that calcium maintains the integrity of the cell membrane and its absence produces "structureless areas," i.e., fragmented membranes, various vesicles, and amorphous inclusions.

Maximum growth for *Caloglossa* occurred over a broader range of potassium concentrations (200 to 400 mg/l) than *Bostrychia* (400 to 500 mg/l). In *Caloglossa* there was a significant increase in growth with increasing concentrations of potassium in the freshwater medium. A similar increase was noted for *Bostrychia*, but only a small percentage of sporelings actually grew. Zimmerman & Steudle (1971) have reported that potassium is significant in turgor regulation and survival in the marine alga, *Chaetomorpha linum*. Similar findings have been reported

for *Pelvetia fastigiata* embryos by Nuccitelli & Jaffe (1976b). In each of these seaweeds, a decrease in osmotic concentration and potassium in the external medium caused a decrease of the potassium concentration internally. However, a decrease in osmolality and increase of the potassium concentration in the external medium caused a slight increase of the potassium internally for *Chaetomorphia linum*. These plants could be maintained in dilute medium for a short time, but prolonged exposure irreversibly damaged the cell due to the large osmotic pressure difference. The observations on growth in this study suggest a similar mechanism may be present in these estuarine algae.

Caloglossa was best able to tolerate the greatest fluctuation in potassium concentrations and even at 0 mg/l it was viable and grew. There was no trace of potassium contamination in the media as determined by atomic absorption spectrophotometry. The growth that occurred suggested either endogenous levels of potassium in the sporelings were sufficient for limited growth and/or potassium may have been replaced by rubidium. Scott & DeVoe (1957) have shown that rubidium replaced up to 66% of the potassium in *Ulva lactuca* within 4 h. Eppley (1960) has observed stimulatory effects of respiration by rubidium and potassium chlorides. Osretkar & Krauss (1965) reported rubidium can replace potassium partially in cell metabolism of the freshwater alga *Chlorella pyrenoidosa*. Substitution of potassium by rubidium caused an 80% decrease in growth. Therefore, potassium can not fully be replaced physiologically.

There were significant interactions between calcium and potassium for both species. The presence of calcium ions was more beneficial to growth at higher levels of potassium for *Bostrychia* (> 300 mg/l) and that of *Caloglossa* (> 200 mg/l). The growth responses for *Bostrychia* and *Caloglossa* were increased at all salinities due to the presence of calcium. The calcium effect was particularly strong for each alga at 15‰ salinity. Eppley & Cyrus (1960) found that *Porphyra perforata* J. Agardh in dilute sea water retained potassium provided that calcium was present. In the absence of calcium, *Porphyra* survived for 30 h in 100% sea water, but only 6 to 8 h in 30% sea water. The loss of potassium in water lacking calcium was thought to be the result of membrane "leakage."

The requirement for calcium and potassium in basic metabolic pathways and ion transport most certainly points to their significance in estuarine waters of reduced salinity. The influx of fresh water does not merely reduce the salinity but also influences the concentrations of the major cations. Water with reduced salinity caused by rainfall and the influx of fresh water by a river could dilute the sea water directly around the algae. The loss of cations through the dilution of sea water, plus its absence in the surrounding water could eliminate membrane selectivity (Eppley & Cyrus, 1960; Manery, 1966) and limit ion-activating respiratory enzymes, e.g., pyruvic kinase (Kjeldson, 1967). The ultimate result of the induced cation loss could be reduced resistance or even death.

The ecological significance of the growth of *Caloglossa* tetrasporelings (and to a

lesser extent *Bostrychia* tetrasporelings) in the freshwater basal medium with 15 mg/l calcium indicates that these benthic marine algae have the potential to penetrate to the freshwater limits of an estuary. Species of *Caloglossa* and *Bostrychia* have been reported in the freshwater limits of many estuaries (Gauthier-Lièvre & Schotter, 1958; Post, 1963a,b,c; 1968). The present study offers a possible mechanism to account for the occurrence of some marine species in fresh water. This study indicates that the horizontal distribution of both species, which is described more fully elsewhere (Yarish & Edwards, 1980), may be accounted for by interactions of salinity, and calcium and potassium. Tetrasporelings of both species grew in the laboratory in the range of salinity, and calcium and potassium variations measured during a single tidal cycle at the two ends of the estuary. However *Bostrychia*, abundant at the mouth and less common at the head of the estuary, grew best at relatively high salinities (25‰) and high levels of potassium (400 to 500 mg/l) in the presence of at least 100 mg/l calcium for maximal growth. *Caloglossa*, abundant in areas of reduced salinity towards the head of the estuary, grew best at a lower salinity of 15‰ and could grow well at fairly low levels of potassium (200 to 400 mg/l) and tolerated extreme reductions in the levels of calcium (2 to 4 mg/l). The abundance of both species thus appears to be a response to a total osmotic effect along the salinity gradient in the Mullica River estuary, since sufficient levels of the major cations were present during the tidal cycle studied. However, perhaps during periods of heavy rainfall and increased freshwater runoff, with concomitant greater dilution of estuarine water, insufficient levels of the major cations would also affect the growth of the two algae at the head of the estuary. Since *Bostrychia* requires a higher salinity and higher levels of calcium and potassium than *Caloglossa* for growth, the critical threshold for the former species would occur first, which may explain its relative rarity towards the freshwater part of the estuary compared to the latter species. Furthermore, the actual upper limit of both species towards the head of the estuary where cation levels fall off rapidly may be determined by low levels of dissolved calcium and potassium in addition to low salinity.

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HETEROTROPHIC BACTERIA ATTACHED TO SEAWEEDS

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Abstract: Viable counts of heterotrophic bacteria attached to the green algae, *Monostroma nitidum* Wittrock and *Enteromorpha linza* (Linné) J. Agardh, ranged from 10^4 to 10^6/cm^2, and those attached to the red alga *Porphyra suborbiculata* Kjellman from 10^3 to 10^4/cm^2. These bacterial populations were larger than those attached to the brown alga *Eisenia bicyclis* (Kjellman) Setchell ranging from 10^1 to 10^4/cm^2. The bacterial populations in the environmental sea water, Nabeta Inlet and Otsuchi Bay (Japan), were 10^3/ml. Orange and yellow pigmented bacteria were predominant on the green and red algae, but not in the bacterial populations of the brown alga and the sea water. Most of the pigmented bacteria were identified as belonging to the *Flavobacterium-Cytophaga* group. A beneficial relationship was suggested between the green algae and the pigmented bacteria. Proportions of *Vibrionaceae* were small on the green algae.

INTRODUCTION

During the last two decades some investigations were made on the heterotrophic bacteria attached to seaweeds. Chan & McManus (1969) reported that the bacterial numbers attached to *Ascophyllum nodosum* and *Polysiphonia lanosa* were 100 to 10 000 times higher than those in the environmental sea water, and that *Vibrio* was predominant on *Ascophyllum nodosum*. Tsukidate (1971) isolated the strains of *Flavobacterium*, *Aeromonas* and *Staphylococcus* from *Porphyra leucostica*. Non-pigmented bacteria which were identified as *Pseudomonas* spp. or *Vibrio* spp. were predominant in the bacterial floras of *Laminaria longicruris*. In the investigations of Chan & McManus and Tsukidate, however, bacterial counts were described as the number per gram dry weight of the algae, despite the fact that bacteria are attached to the surface of the algal thalli. The bacterial floras were examined with the small numbers of bacterial isolates. In the investigation of Laycock (1974) no comparison was made between the bacterial floras on the seaweed and those of the environmental sea water. Hence there still remains unsolved the question whether a specific taxonomic group of bacteria is characteristic of seaweeds. In this report the bacterial populations and floras of the green algae, *Monostroma nitidum* Wittrock and *Enteromorpha linza* (Linné) J. Agardh, and the environmental sea water are described. Also

the bacterial numbers on the red alga *Porphyra suborbiculata* Kjellman and the brown alga *Eisenia bicyclis* (Kjellman) Setchell are reported.

Materials and Methods

COLLECTION OF MATERIALS

Seaweed and sea-water samples were collected in the period from December 1973 to June 1974 at Nabeta Inlet, Shizuoka prefecture (34°40′N : 138°55′E). Only *Enteromorpha linza* and sea water were collected in November 1979 at Otsuchi Bay, Iwate prefecture (39°21′N : 141°56′E). Nabeta Inlet is ≈1 km in length and 0.2 km in width. The collection site is located in the central part of it. Polluting effects of a small inflow from land have some effect. Water temperature during the period of collection ranged from 20.6 °C in June to 11.4 °C in February. Otsuchi Bay is ≈8 km in length and 2 km in width. There are three rivers inflowing at the deepest part of the Bay. Polluting influences are effective only to a small extent, because the collection site is 4 km from them. Salinity was usually >33‰, and water temperature at the time of collection was 18.6 °C. The high-tidal seaweed samples, *Enteromorpha linza*, *Monostroma nitidum* and *Porphyra suborbiculata*, were collected at low tide when water level was not under the seaweeds. In case of the brown alga *Eisenia bicyclis*, middle-aged leaves were collected at low tide from a labelled individual, because different bacterial populations, 10^5 to 10^0 cells/cm^2, depending upon the growing ages of the leaves were observed in a preliminary experiment (data not included). Sea-water samples were collected near the collection sites at the depth of 1 m at high tide using a JZ sampler (ZoBell, 1946).

ENUMERATION OF BACTERIA

Seaweed samples were rinsed with sterile sea water to remove the bacteria originating from the environmental sea water. The washed seaweeds were cut with a sterile corkborer of 11.4 mm in diameter to give five 1-cm^2 pieces. After being washed with sterile sea water again, these five pieces were blended for 3 min with 30 ml of sterile sea water containing 1 mg/l of Tween 80. During the blending treatment, the blending jar was iced to prevent the bacteria from heat damage. Ten-fold dilutions of seaweed and sea-water samples were spread on PPES-II plate (Taga, 1968) which contained (in g per 1000 ml of 90% aged sea water): Polypepton (Daigo Co.), 2.0; Soytone (Difco), 1.0; yeast extract (Difco), 1.0; proteose peptone no. 3 (Difco), 1.0; ferric citrate, 0.1; agar, (Difco), 15.0. The pH was adjusted to 7.6. The plates were incubated at 20 °C for 10 days before colonies were counted.

IDENTIFICATION OF BACTERIA

Bacterial strains were randomly isolated from the plates, and were cultured using PPES-II medium. Twenty-four-hour slope cultures were Gram-stained using Hucker's modification (Conn *et al.*, 1957). Motility was examined with hanging drop preparations of 24 h broth cultures at 20 °C. In the case of strains isolated from the samples collected at Otsuchi Bay, hanging drop preparations of 24 and 48 h, and 1- and 3-wk slope cultures at 20 °C, and also those of 1-wk slope cultures at 10 °C were examined. The shapes of cells were noted using 24-h broth cultures under a phase microscope. Air-dried preparations of 24-h broth cultures were flagella-stained using a modified Bailey's method (Fisher & Conn, 1942). Acid production from glucose was tested using the method of Hugh & Leifson (1953) with the following modification: all the ingredients without NaCl were dissolved in a synthetic sea water formulated by Lyman & Fleming (1940). Oxidase activity was examined using the method of Kovács (1956). Catalase activity was examined using 3% H_2O_2. The bacterial strains were identified according to the scheme of Simidu (1974), which is a slightly modified scheme of Shewan *et al.* (1960).

EXAMINATION OF BACTERIAL PIGMENT

Existence of bacteriochlorophyll *a* was examined by a spectrophotometric method using the methanol extracts of bacterial cells, because we recently isolated aerobic photopigmented bacteria from *Enteromorpha linza* (Shiba *et al.*, 1979). Although the photopigmented bacteria were motile by means of polar or sub-polar flagella, the other characteristics were very similar to *Flavobacterium*.

RESULTS

Table I summarizes the bacterial populations on the surfaces of the seaweeds and in their environmental sea water. Viable counts of heterotrophic bacteria attached to the green algae, *Monostroma nitidum* and *Enteromorpha linza*, ranged from 10^4 to $10^6/cm^2$, and those on the red alga *Porphyra suborbiculata* ranged from 10^3 to $10^4/cm^2$. The counts of sea-water samples were 10^3/ml. These bacterial populations remained relatively constant throughout the survey period of 7 months at Nabeta Inlet. In the case of *Enteromorpha linza* and sea-water samples, there was no significant difference between Nabeta Inlet and Otsuchi Bay. The bacterial populations of the brown alga *Eisenia bicyclis* were generally smaller than those of the green and red algae, and fluctuated considerably; the largest number of bacteria ($2.2 \times 10^4/cm^2$) was obtained in March, and the smallest ($2.9 \times 10^1/cm^2$) in January. This fluctuation of bacterial numbers was not influenced by the variation of the bacterial populations in sea water where the populations remained relatively constant, but it was considered to be dependent on the physiological condition of the seaweed. The attached bacterial population was the smallest when the largest number of the germinating leaves was observed (Fig. 1).

The bacterial populations of the green and red algae examined were generally characterized by the predominance of yellow and orange pigmented bacteria (Table II). The proportions of the pigmented bacteria on these algae ranged from 72.4 to 15.4%, while those on the brown alga *E. bicyclis* ranged from 18.8 to 0.0%, and those in the sea water from 16.7 to 0.0%.

TABLE I

Viable counts of heterotrophic bacteria attached to seaweeds and those in sea water: bacterial counts of seaweeds are described as the number per cm^2, and those of sea water are the number per ml.

Samples	Collected at							
	Nabeta Inlet (1973 to 1974)							Otsuchi Bay (1979)
	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Nov.
Monostroma nitidum	3.4×10^6	3.1×10^5	4.8×10^5	4.5×10^5	1.5×10^5	1.0×10^6	5.6×10^5	–
Enteromorpha linza	–	–	3.2×10^4	1.1×10^5	8.4×10^4	6.3×10^5	–	1.4×10^5
Porphyra suborbiculata	8.8×10^3	–	3.6×10^3	5.2×10^4	3.3×10^3	1.7×10^4	–	–
Eisenia bicyclis	2.2×10^3	2.9×10^1	8.2×10^2	2.2×10^4	7.6×10^3	1.8×10^3	1.2×10^3	–
Sea water	3.6×10^3	2.8×10^3	1.7×10^3	1.8×10^3	2.3×10^3	4.1×10^3	3.3×10^3	2.8×10^3

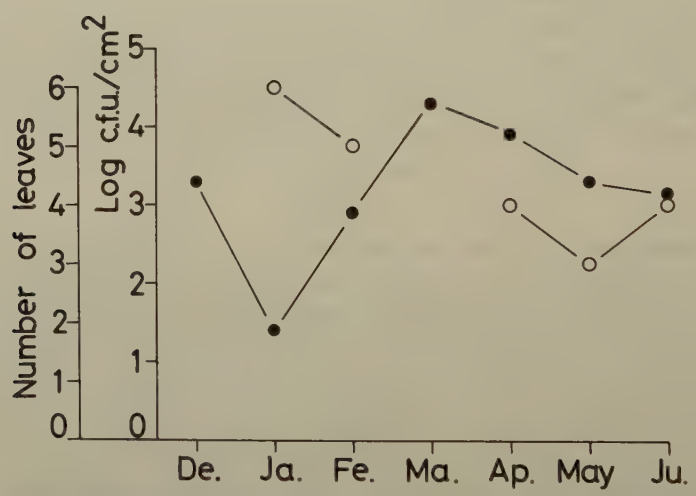

Fig. 1. Relationship between the numbers of heterotrophic bacteria attached to middle-aged leaves of a labelled *Eisenia bicyclis* and the numbers of germinated leaves during the collection intervals: ●, viable counts of bacteria; ○, numbers of germinated leaves.

Fig. 2 summarizes the bacterial floras of the green algae and their environmental sea water. In the sea-water samples, Gram-negative, pleomorphic, non-pigmented and non-motile bacteria were predominant. These bacteria were assigned to the genus *Acinetobacter* according to the scheme of Simidu (1974), but the bacteria

showed a specific property of *Moraxella*, i.e., they were oxidase-positive. *Vibrionaceae* were predominant in the sea water of Otsuchi Bay, but not in that of Nabeta Inlet. The bacterial populations of the green algae were generally characterized by the predominance of *Flavobacterium-Cytophaga* which was non-motile and showed no spreading growth on PPES-II agar. The percentages of *Flavobacterium-Cytophaga* were, however, relatively small compared to those of the pigmented bacteria, because many pigmented bacteria died during serial sub-cultures. Bacteriochlorophyll *a* was obtained from only one strain isolated from *Enteromorpha linza* of Otsuchi Bay. Motility could not be observed. None of the strains of Nabeta samples was

TABLE II

Percentages of yellow and orange pigmented bacteria in the bacterial floras of seaweeds and sea water.

Samples	Collected at							
	Nabeta Inlet							Otsuchi Bay
	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Nov.
Monostroma nitidum	20.3	53.0	–	68.3	65.1	72.1	30.0	–
Enteromorpha linza	–	–	42.0	46.7	53.4	35.7	–	72.4
Porphyra suborbiculata	53.7	–	61.2	69.0	36.7	15.4	–	–
Eisenia bicyclis	7.0	0.0	11.1	0.0	12.5	18.8	18.0	–
Sea water	1.0	0.0	7.0	9.5	8.9	9.7	6.0	16.7

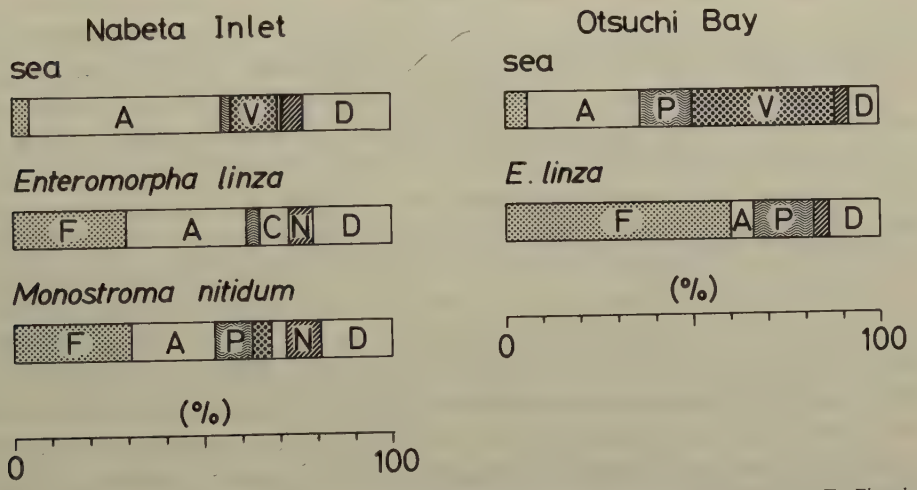

Fig. 2. Generic compositions of the bacterial populations on seaweeds and in sea water: F, *Flavobacterium-Cytophaga*; A, *Acinetobacter*; P, *Pseudomonas*; V, *Vibrionaceae*; C, coryneform bacteria; N, not identified bacteria; D, dead strains; the numbers of bacteria examined are as follows: sea water, 90 (April to June 1974 at Nabeta) and 50 (November 1980 at Otsuchi); *Enteromorpha linza*: 60 (April to May at Nabeta) and 50 (November at Otsuchi); *Monostroma nitidum*, 70 (April to June at Nabeta).

examined for the pigment. Although *Acinetobacter* was also predominant on the seaweeds of Nabeta Inlet, it was not predominant on *Enteromorpha linza* collected at Otsuchi Bay. It is interesting to note that there was a very small proportion of *Vibrionaceae* on the green algae.

Discussion

Densities of heterotrophic bacteria attached to the seaweeds examined were different between algal species, i.e., the bacterial numbers on the green algae, *Monostroma nitidum* and *Enteromorpha linza*, were 10^4 to $10^6/cm^2$, and those of the red alga *Porphyra suborbiculata* were 10^3 to $10^4/cm^2$. These bacterial populations were generally larger than those on the brown alga *Eisenia bicyclis* ranging from 10^1 to $10^4/cm^2$. With regard to other brown algae, small bacterial numbers have been reported. Chan & McManus (1969) reported 10^4 to 10^7/g dry wt of *Ascophyllum nodosum*. Conover & Sieburth (1964) reported 10^1 to 10^5 cells/0.5 g dry wt of *Sargassum natans*. The bacterial numbers on healthy parts of *Laminaria longicruris* were 10^1 to $10^3/cm^2$ (Laycock, 1974). Although the small bacterial populations on the brown algae are considered to be due to the organic substances released from the algae, Sieburth (1969) reported that the rates of release of organic materials from brown algae were not characteristically lower than those from green algae. The rates of release from fast growing seaweeds were higher than those from the slow growing individuals (Khailov & Burlakova, 1969). In this experiment the smallest bacterial number on *Eisenia bicyclis* was found in January when the alga seemed to grow most rapidly (Fig. 1). Hence it appears that the bacterial populations on the brown algae, especially *E. bicyclis*, are not depending on the quantity of the released materials of the algae. Craigie & McLachlan (1964) reported that in the seaweeds examined only brown algae excrete yellow, UV-absorbing materials which appear to be flavonol or catechin-type tannins. The inhibition of bacteria by tannic acid and tannin-like substances extracted from *Sargassum natans* was reported by Sieburth & Conover (1965). The small bacterial populations on *Eisenia bicyclis* appear to reveal antimicrobial activity of the organic material released by the alga.

On the other hand, the existence of large and constant bacterial populations on the green algae *Monostroma nitidum* and *Enteromorpha linza* suggests that there exists a beneficial relationship between the green algae and their epiphytic bacteria. Although *Acinetobacter* was predominant on the seaweeds collected at Nabeta Inlet, it was also predominant in their environmental sea water and not predominant on *Enteromorpha linza* collected at Otsuchi Bay. The bacteria belonging to the *Flavobacterium-Cytophaga* group were, however, characteristically predominant on the green algae collected at both stations, and not predominant in their environmental sea water. Hence it is possible that a beneficial relationship exists between the green algae and their epiphytic bacteria belonging to the *Flavobacterium-*

Cytophaga group. Although there still has been no report on the relationship between seaweeds and *Flavobacterium-Cytophaga*, Sieburth (1968) reported that the populations of *Flavobacterium* in sea water peaked in the period of maxima of photosynthetic potential of phytoplankton. Growth of *Flavobacterium* strains was found to be stimulated by *Skeletonema costatum* by Kogure *et al.* (1979).

It is noticeable that the proportion of *Vibrionaceae* was small in the bacterial floras on the seaweeds examined (Fig. 2). *Vibrionaceae* is generally predominant in the sea water of open sea around Japan (Simidu *et al.*, 1977), and was also predominant in the environmental sea water of Otsuchi Bay (Fig. 2). The inhibition of *Vibrio* growth in the presence of phytoplankton was reported by Simidu *et al.* (1977). The decreasing trend of *Vibrio* population during the maxima of photosynthetic potential of phytoplankton was also reported (Sieburth, 1968). It might be suggested that the seaweeds examined had an inhibitory effect on the development of *Vibrionaceae* populations on their surface.

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ABNORMAL GROWTH PATTERNS IN THE SEA URCHIN *TRIPNEUSTES* CF. *GRATILLA* (L.) UNDER POLLUTION (ECHINODERMATA, ECHINOIDEA)

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Abstract: Two populations of deformed sea-urchins were found in the vicinity of a combined power and desalination plant in the Gulf of Eilat (Aqaba), Red Sea. This area is highly polluted by thermohaline and heavy metal ion effluents. More than 60% of the urchins showed irregular bulging of the aboral half of the test. The height (H) to diameter (D) ratio for the most affected population was 0.70 ($\pm$0.15), compared with 0.53 ($\pm$0.04) for a normal population, away from the power plant. In extremely deformed individuals the H/D ratio reached 1.4. The deformed sea-urchins had a wider peristome aperture and larger Aristotle's lantern, and fewer interambulacral plates than a normal urchin of the same diameter.

Growth rate of highly deformed urchins in aquaria, under non-polluted conditions, was very low. In 2 months their average diameter increased by only 0.2 mm, compared with 7–8 mm in normal or slightly deformed urchins, under the same conditions.

Growth lines in the deformed urchins' plates indicate excessive growth in the meridional direction which is normally much less. It is suggested that controlled growth was disrupted. Some possible controlling mechanisms are discussed.

INTRODUCTION

The sea-urchin skeleton consists of close-fitting calcium carbonate plates that are added in the apical region. Differential plate growth accounts for the definite form that is characteristic to each species. The morphology of the echinoid test and its variation has been studied by Lovén (1871, 1875), Deutler (1926), Jensen (1969), Pearse & Pearse (1975), and Märkel (1975, 1976). Raup (1968) discussed the test shape and plate patterns and formed a computer simulated model that explains the echinoid growth and plate configuration as a result of two main factors, plate supply rate and the gradient that affects the rate of meridional growth. A wide variety of plate configurations can be achieved by changing these factors.

The biometrical relations in regular sea-urchins have been documented (Mortensen, 1943; Regis, 1969, 1973; Märkel, 1975) and abnormalities in the shape of the echinoid test have been discussed by Koehler (1924) and Jackson (1927) and reviewed by Swan (1966). Additional data on aberrant growth are given by Moore (1974), according to whom many abnormalities result from previous injury and ensuing imperfect regeneration, but others result from metabolic disturbances.

Other abnormal phenomena involve deviant skeletal proportions, especially the

relations between test height (H) and ambital diameter (D). Mortensen (1943) observed and illustrated abnormal individuals of *Salmacis bicolor* and *Holopneustes inflatus* with exaggerated H/D ratio resulting from parasitic infection by a snail. Ernst (1973) calculated H/D ratio (L/H in his terminology, which is the reciprocal of H/D) for *Arbacia lixula* and *Paracentrotus lividus* and found that different populations of the same species may have different H/D due to possible environmental factors. Allain (1978) reported a large concentration of deformed *Lytechinus variegatus* from Cartagena Bay, Colombia. About 5% of a total of more than 2000 urchins had skeletal deformations. He believed that oil and wastewater pollution caused the deformations.

Tripneustes cf. *gratilla* (L.) is a large and common sea-urchin in the northern Red Sea. It lives subtidally on protected rocky beaches and reef flats (James & Pearse, 1969). Contrary to other Indo-Pacific localities, in the Red Sea this species prefers the algal covering of boulders and rarely occurs in sea-grass beds, as reported for Indo-Pacific *T. gratilla* (Lawrence, 1975). According to T.A. Ebert (pers. comm.) and data collected by the author, the Red Sea *Tripneustes* differs from the Indo-Pacific *T. gratilla* in some aspects and its taxonomy needs revision. It will hereafter be referred to as *T.* cf. *gratilla*.

In this paper two highly deformed populations of *T.* cf. *gratilla*, probably due to acute pollution, are described. The biometry and the possible deformation mechanisms are discussed.

Material and Methods

AREA STUDIED

The most severely polluted area in the Gulf of Eilat (Aqaba) is near the Eilat harbour, in the vicinity of a combined power station and desalination plant (Fig. 1). The discharged water effluent is thermohaline (39 °C and $S = 46‰$) and shows high concentrations of dissolved copper and iron (Dafni, 1974a,b). Occasionally, during cleaning of the pipe system, acidic water is discharged, which may contain up to 70 mg/l dissolved iron and 30 mg/l copper as well as residual organic compounds, applied to reduce calcite precipitation in the system. Hydroxides of iron and copper precipitate in the sea around the outlet (Lev-er *et al.*, 1976). As the effluent is denser than sea water, it spreads along the bottom and is carried southwards by the prevailing southerly surface current. This effluent affects the sublittoral fauna for a distance of 200 m to the south, and to a depth of 12 m (Dafni, 1974a). It creates an almost sterile area, 70 m wide, opposite the discharge point, only invaded by fish schools. The conditions improved 2 yr ago when measures were applied to reduce the pollution. The sea-urchin populations now being studied were probably established following this improvement.

From the outlet for a distance of 200 m southwards there are suitable substrata

for sea-urchins. From 200 m to 1000 m southwards the beach consists of non-consolidated gravel, which does not offer a suitable substratum for sea-urchins. Northwards and further than 1000 m southwards the beach consists of boulders and beachrock and is populated by sea-urchins.

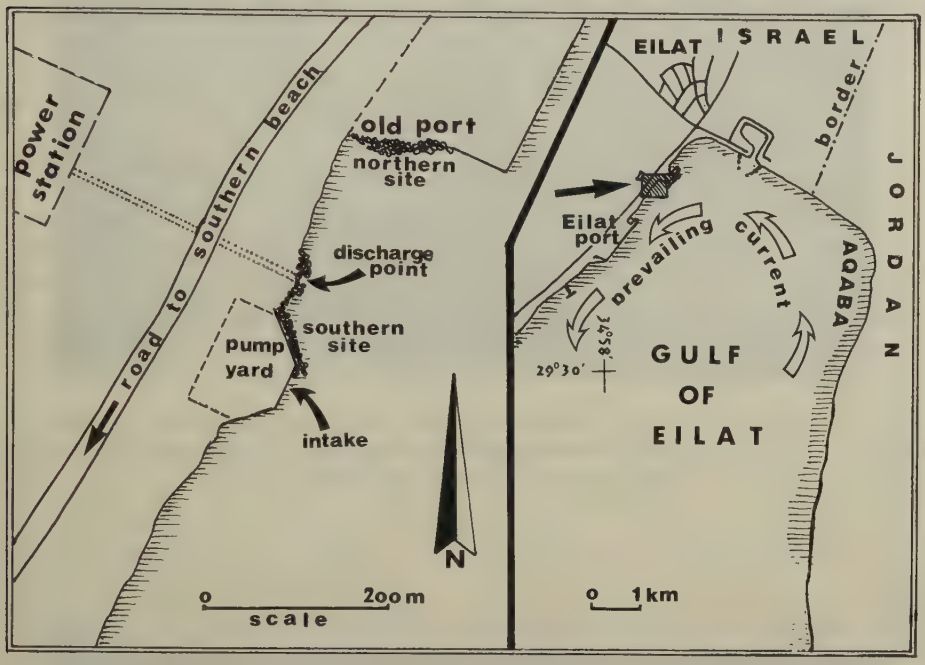


Fig. 1. Map of the northern Gulf of Eilat (Aqaba), showing the polluted area and the location of the affected populations, the northern and southern sites: the non-affected sea-urchins were collected from the southern beach, not included in the map.

MATERIAL

Two populations of *T.* cf. *gratilla*, showing a high degree of skeletal deformation, were found in the subtidal zone at a depth of 1–3 m on either side of the discharge point (Figs. 2, 3). The first population was found 60–120 m south of the discharge point. Until 11 May, 1979, when an acid spill killed all the sea-urchins in this locality, dense populations of the three common species were found here: *Echinometra mathaeii* (Blainville), *Diadema setosum* (Leske), and *Tripneustes* cf. *gratilla*. Only the third species showed such deformations. The second *Tripneustes* population was living on boulders bordering the old port, only 150 m north of the discharge point. The two populations were separated by a sterile zone.

For the purpose of comparison, urchins were also examined at other localities, 1–12 km south of the power station.

Fig. 2. Two living *Tripneustes* cf. *gratilla* with the same ambital diameter (27 mm): right, a highly deformed sea-urchin from the southern site, with H/D ratio of ≈1.3; left, a normal urchin from a non-polluted environment, with H/D of 0.53.

Fig. 3. Three skeletons from the southern population, demonstrating the bizarre shapes of the deformed urchins.

FIELD OBSERVATIONS AND MEASUREMENTS

Test ambital diameter (D) and test height (H) of 117 randomly collected living individuals of *T.* cf. *gratilla* from the southern population, 95 from the northern population, and 139 from other localities were measured using venier calipers with a precision of 0.1 mm. For an estimate of the diameter, the average of five measurements for each individual was used. The single largest value was used in height. The H/D ratio was calculated in order to demonstrate abnormalities in the test shape.

LABORATORY GROWTH EXPERIMENTS

Living sea-urchins were transferred to tanks with running water in the H. Steinitz Marine Laboratory under non-polluted conditions. The water temperature in the tanks varied between 24–26 °C, the ambient sea-water temperature in summer. The sea-urchins fed by scraping encrusting algae off stones that were frequently supplied.

The following groups of sea-urchins were kept in different tanks during a period of 2 months, from 27 May to 27 July, 1979.

(1) Group 1, 10 highly deformed sea-urchins from the southern population, with a mean H/D ratio of 1.02 at the beginning of the experiment.

(2) Group 2, 29 sea-urchins from the northern population, only slightly deformed, with an initial mean H/D ratio of 0.60.

(3) Group 3, 9 urchins from a non-polluted area (near the Marine Laboratory), with an initial H/D ratio of 0.53.

After the final measurements most of the animals were maintained in the tank and further data were recorded.

BIOMETRICAL DATA

Two days after the lethal spill on 11 May, 1979, ≈300 skeletons of *Tripneustes* from the southern population were collected at random. Measurements of test diameter, height, peristome and Aristotle's lantern were made together with counts of plates and other observations for a biometrical analysis.

PLATE MICRO-STRUCTURE

Growth lines of single plates, whole ambulacral (A) and inter-ambulacral (IA) plate rows of normal and deformed sea-urchins were examined following the technique described by Pearse & Pearse (1975). Cleaned plates were charred for 10–20 min at 300–350 °C in a furnace and then immersed in xylene and embedded in Canada Balsam. Since Red Sea *Tripneustes* has only a few small spine tubercles no grinding of the plates was necessary for skeletons up to a diameter of 50–60 mm.

RESULTS

FIELD OBSERVATIONS AND MEASUREMENTS

Measurements of H/D ratio of living *T.* cf. *gratilla* are shown in Table I. The population from the most polluted southern site showed an abnormally high H/D,

TABLE I

Test height to diameter ratio (H/D) for sea-urchins from various populations, mentioned in the text: N = number measured.

Population	N	Mean H/D and SD
Southern population	117	0.70 (± 0.15)
Northern population	95	0.59 (± 0.06)
Other localities	139	0.53 (± 0.04)

up to 1.40 in individual cases. In this work only urchins with H/D >0.6 are considered abnormal or deformed. The mean H/D ratio for sea-urchins of the southern population was 0.70 with a high SD (0.15). The northern population showed a lower mean H/D (0.59) and contained a smaller number of deformed urchins. Since the H/D ratio varied differently in the populations, a non-parametric analysis (Kruskal & Wallis, 1952) was applied to test the difference between the H/D ratios of the populations. It was found to be significant at $P < 0.01$. Fig. 4 shows the

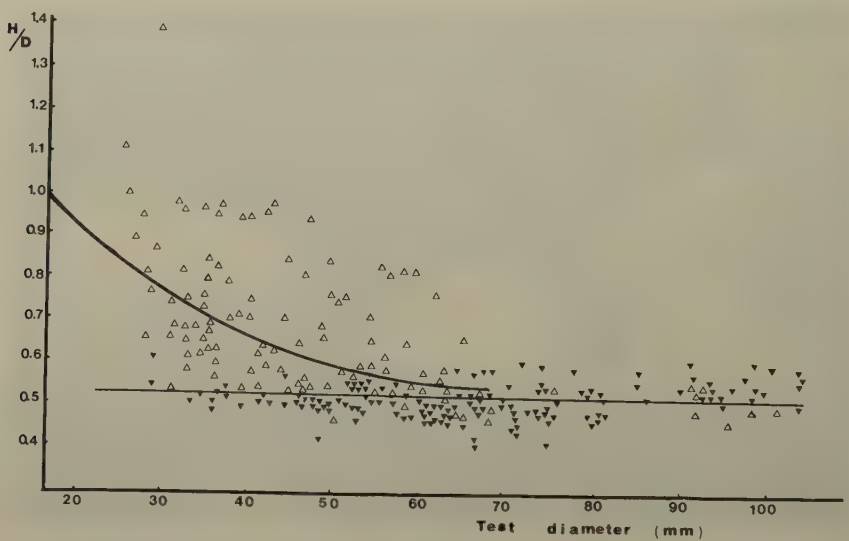


Fig. 4. The relation between H/D ratio and test diameter for 117 sea-urchins from the southern population (▼) and 139 from other, non-polluted localities (△).

relation between test diameter and H/D of the southern population and sea-urchins from non-polluted localities and a wide range of individual H/D values.

The correlation between H/D as a function of diameter for the southern population was $r = 0.57$, which is significant at $P < 0.05$. Urchins from non-polluted localities showed a constant H/D ratio at all sizes, although a small increase is noted for urchins >70 mm. The regression slopes merged at ≈70 mm. From Table II it may be seen that in the southern population up to 94% of the urchins

TABLE II

Relative frequency (%) of the H/D ratio for various size groups in urchins from the southern population: N = number measured.

Test diameter (mm)	N	Non-deformed (H/D = 0.4–0.6)	Slightly deformed (H/D = 0.6–0.8)	Highly deformed (H/D > 0.8)
25–35	33	6.1	54.5	39.4
35–45	27	18.5	48.1	33.3
45–55	21	33.3	47.6	19.0
55–65	22	50.0	36.4	13.6
>65	14	100.0	0	0
Entire population	117	33.3	41.9	24.8

at the size of 25–35 mm showed abnormal H/D, while at >65 mm all the urchins had normal H/D ratio. Irregularities not expressed in H/D ratio were, however, not uncommon in larger urchins' tests.

Similar results were observed also for the northern population, although not presented here.

GROWTH EXPERIMENTS

Results from the growth experiments are summarized in Table III. Group 1 (highly deformed sea-urchins from the southern population) showed no significant

TABLE III

Diameter size (mm) and H/D change in urchins from various populations during 60 days in non-polluted conditions: for key to groups see text; N = number measured.

Group	Population	Initial measurements (27 May)			Final measurements (27 July)		
		N	Diameter ± SD	H/D ± SD	N	Diameter ± SD	H/D ± SD
1	Southern	10	36.84 (9.08)	1.02 (0.17)	10	37.06 (9.36)	1.05 (0.18)
2	Northern	29	37.53 (7.04)	0.60 (0.07)	29	44.64 (5.26)	0.59 (0.05)
3	Marine laboratory	9	39.67 (6.30)	0.53 (0.01)	7	47.25 (3.65)	0.54 (0.03)

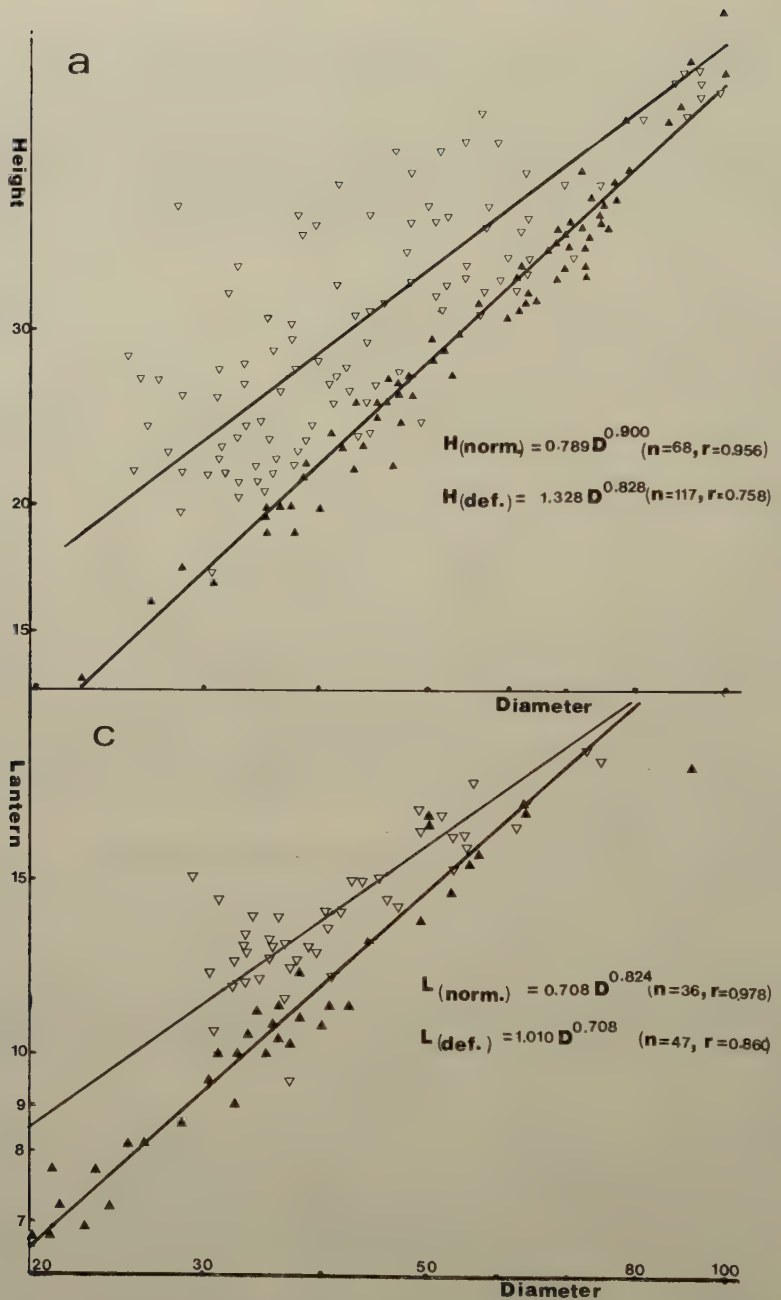
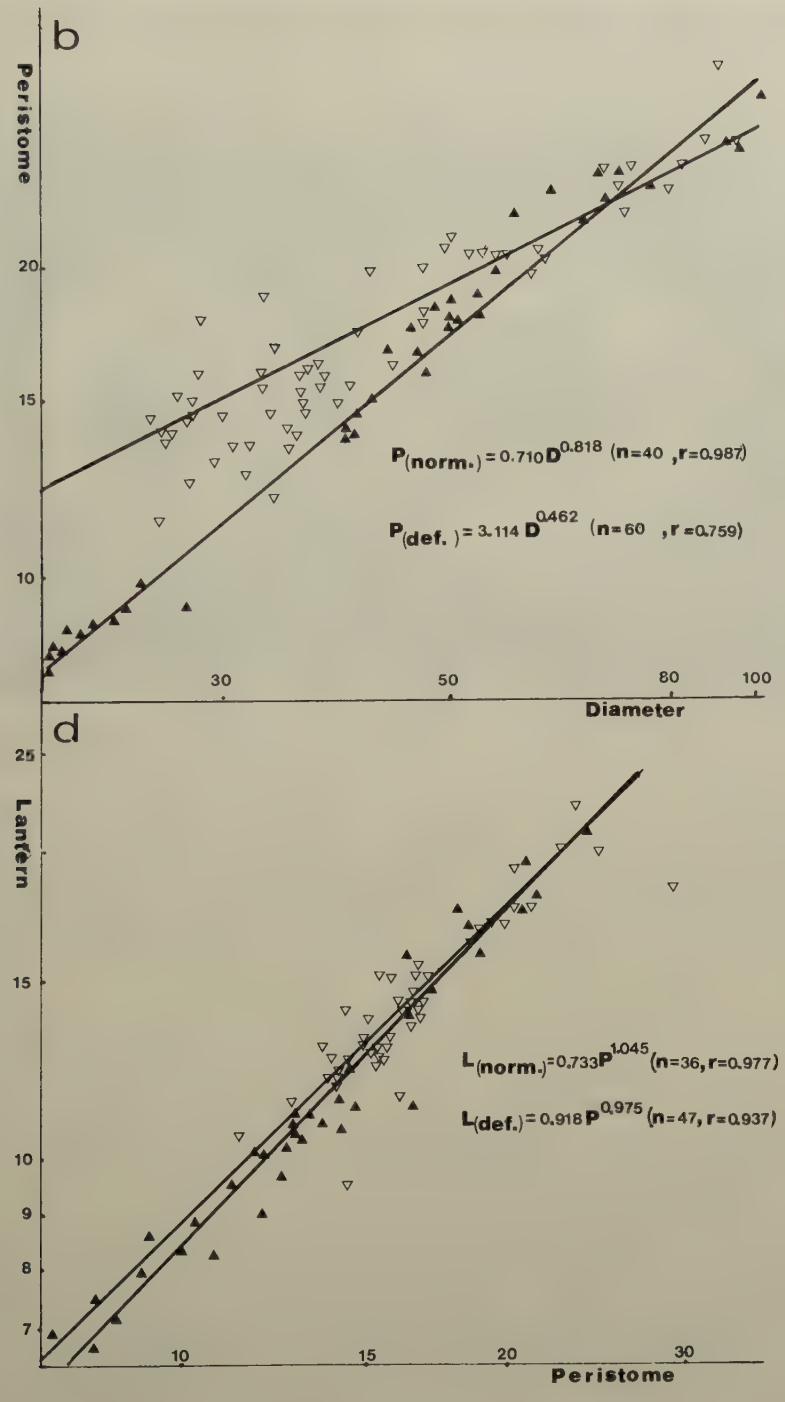


Fig. 5. Regression lines for the allometrical relations (log scale) between diameter (D), height (H), peristome (P), and lantern (L) for sea-urchins from the southern population (▽) and from other, non-polluted localities (▲): all sizes are in mm.


b
Peristome
20
15
10
30
50
80
100
Diameter
P(norm.) = 0.710 D^0.818 (n=40, r=0.987)
P(def.) = 3.114 D^0.462 (n=60, r=0.759)
d
Lantern
25
15
10
9
8
7
10
15
20
30
Peristome
L(norm.)=0.733P^1.045 (n=36, r=0.977)
L(def.)=0.918P^0.975 (n=47, r=0.937)

growth in test diameter (t-test, $P > 0.5$) even after removal from the polluted environment. (This result was also confirmed by further measurements. On 4 October, 4.5 months after the experiment had started, Group 1 urchins had a mean diameter of 37.7 mm, an average increase of only 1 mm.) The mean H/D ratio of the test of these animals increased, non-significantly, from 1.02 to 1.05. Group 2 (less deformed urchins from the northern population), showed a significant increase in diameter, similar to the growth rate observed in the non-deformed population (Group 3) ($P < 0.05$). The H/D did not change in either of the groups.

BIOMETRICAL DATA

The biometrical relations between various parts of an animal's body are often described by the allometry equation (Teissier, 1960):

$$Y = AX^B \tag{1}$$

or by its logarithmic transformation

$$\ln Y = B\ln X + \ln A \tag{2}$$

B, the exponent, or slope of equation (2) was used by Regis (1969, 1973) as the allometry constant (α), while $\ln A$ ($\ln Y$ when $\ln X = 0$) is the initial growth index.

The allometrical constants for the relations between ambital diameter and height, peristome and Aristotle's lantern diameters were calculated for sea-urchins from the southern population, deformed and non-deformed, and for urchins from non-polluted localities (Fig. 5). It was suggested by Ricker (1973) that in regressions, in which both categories are measurements subjected to errors, the use of the functional regression slope, B/r, is more suitable for the description of the relationship between them (r is the correlation coefficient). A covariance analysis (Snedecor & Cochran, 1967) was used to test the homogeneity, the differences between the allometrical constants (slopes) and the elevations for each pair.

From Fig. 5 it can be seen that the allometrical proportions for a normal population are similar. Nevertheless, some noticeable differences should be mentioned. Regression slope of peristome-diameter (Fig. 5b) for normal urchins is flatter than the slope of height-diameter (Fig. 5a). This is mainly due to the lesser expansion of the peristome compared with the diameter growth. It was found that for a young normal urchin (D = 10 mm) the peristome/diameter ratio is 0.45, and for an old urchin (D = 70 mm) it is only 0.3.

The isometric lantern-peristome growth in normal urchins can be seen from their similar slopes (Figs. 5b, c) ($B/r = 0.824$ and 0.818, respectively) and from Fig. 5d. As for the deformed urchins, except for the afore-mentioned excessive height to diameter ratio (Fig. 5a) a large peristome (54% of the test diameter) is shown (Fig. 6). Peristome-diameter slope (Fig. 5b) is lowest ($B/r = 0.462$). The lantern of a deformed urchin is also significantly larger than shown for the normal (Fig. 5c),

although not as large as the peristome (Fig. 5b). Lantern to peristome growth was, however, found to relate isometrically for both populations (Fig. 5d).

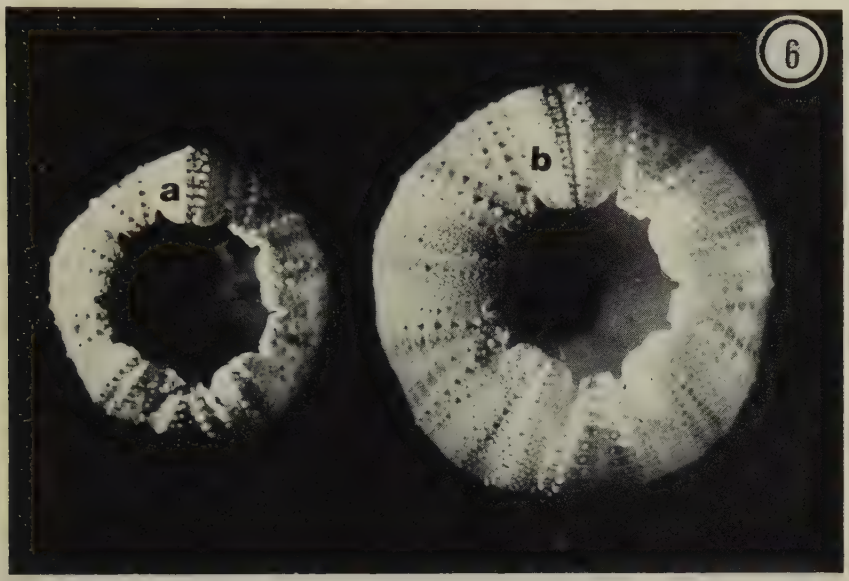


Fig. 6. Oral view of a deformed (a) and a normal (b) sea-urchin with the same peristome size (15 mm): peristome/test diameter ratio for the normal urchin is 0.38 and for the deformed urchin it is 0.54.

Since covariance analysis between groups assumes equal homogeneity within each pair, the high variance of the deformed population's data rendered this analysis non-significant for Figs. 5a, b, c. In the light of the observation that highly deformed urchins stop growing in the horizontal axis (Table III), for a considerable time, the last mentioned facts may imply that peristome and lantern growth continued unaffected.

NUMBER OF INTERAMBULACRAL PLATES

In all regular sea-urchins the number of interambulacral and ambulacral plates increases with age and is proportionally related to size. Fig. 7a shows counts of the IA plates of normal *T.* cf. *gratilla* from non-polluted localities and from the southern population, plotted against their test diameter. Normal urchins showed a straight line for animals up to 70 mm, >70 mm the slope was steeper. This last-mentioned observation will be discussed below. Southern population urchins, both with abnormal and normal H/D ratio formed a significantly different slope ($P < 0.01$). Thus, small deformed urchins had 1–2 fewer IA plates than normal animals of the same diameter size.

For deformed sea-urchins diameter is a poor indicator of age, because of its

much reduced growth rate. In this case the peristome may show a better correlation with age, in the light of its presumed unaffected increase. Therefore, IA plate number was plotted against peristome size (Fig. 7b). It appears that small deformed urchins show 3 4 fewer plates than normal urchins with the same peristome size ($P < 0.01$).

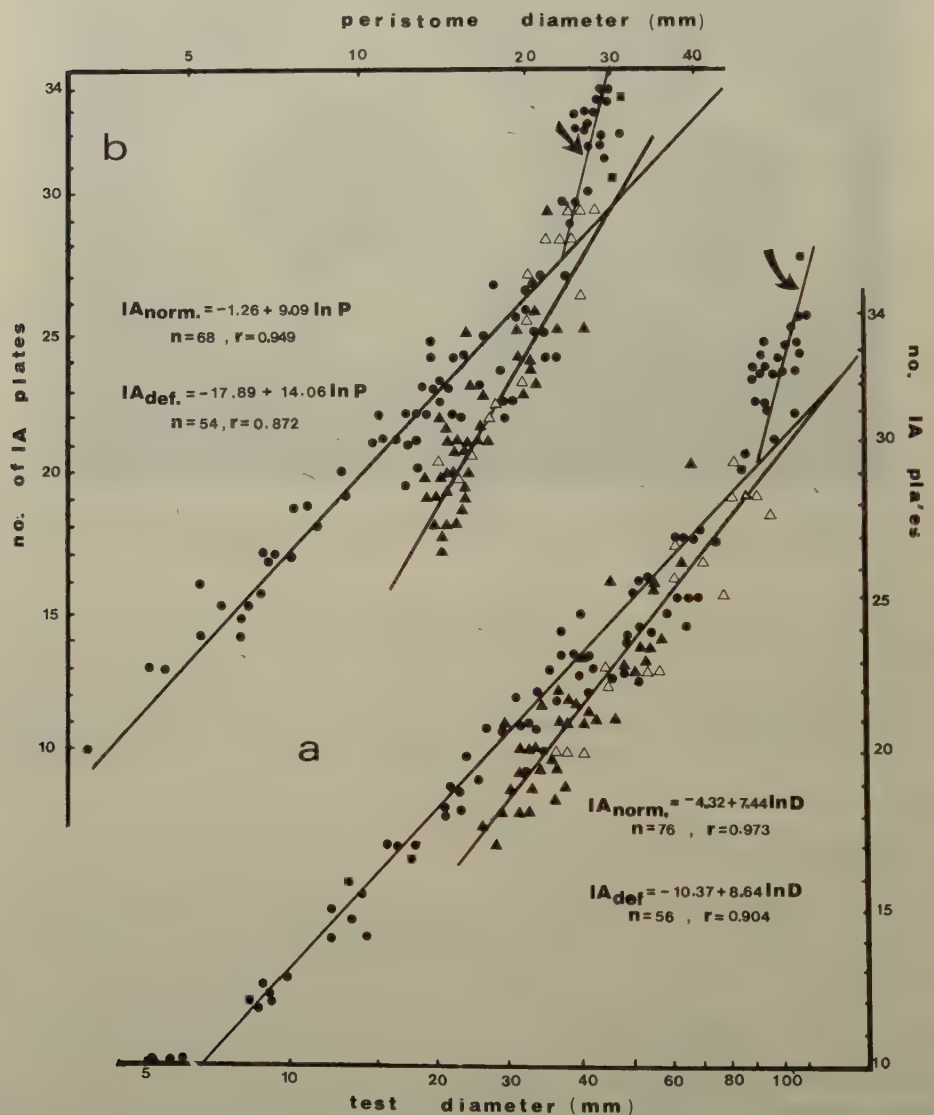


Fig. 7. Relation between number of interambulacral plates and (a) test diameter or (b) peristome diameter, for urchins with normal (△) and abnormal (▲) H/D ratio from the southern population, and for urchins from non-polluted populations (●): arrows point to a tendency shown by normal larger urchins to add IA plates with only a slight increase of the diameter.

OTHER TEST DEFORMATIONS

In addition to the abnormalities mentioned above the skeletons of deformed urchins collected in the polluted area showed many other irregularities.

(1) There were irregularities in the shape and symmetry of IA plates, mostly in the aboral region. Some plates had secondary sutures that divided them, while other IA plates had their latitudinal sutures twisted and joining a parallel suture (Fig. 8). Some plates were completely fused with the neighbouring plates by omission of one or several sutures.

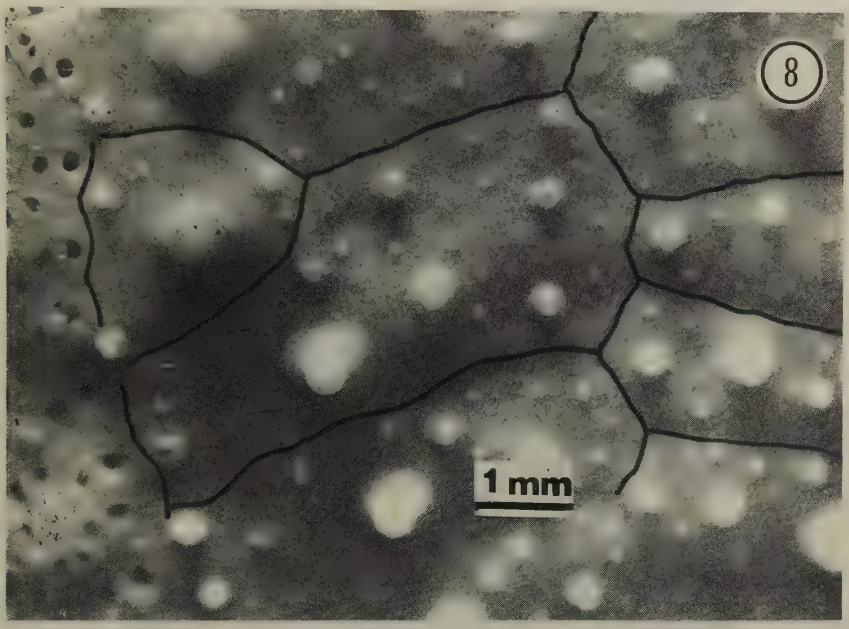
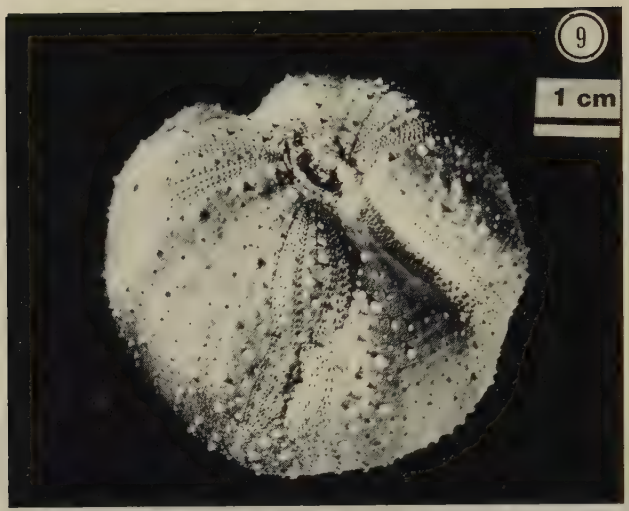
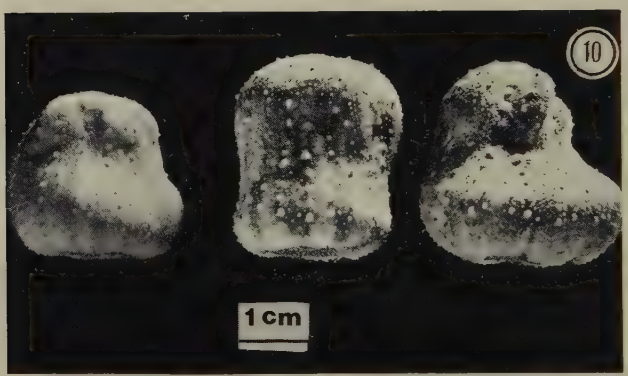


Fig. 8. Interambulacral plates of a highly deformed sea-urchin, showing a latitudinal suture bending and joining a parallel suture, while the adjacent plate meets in the interradial suture 3 plates: to the left, the irregular shape of the ambulacral plates can be seen (suture lines were drawn over with Indian ink).

(2) Ambulacral plates were often deformed and the pore rows were shifted in their position towards each other (Fig. 9), described by Moore (1974) as "pinching". This phenomenon was common and was also observed in older sea-urchin tests that did not show an abnormal H/D ratio. Pinching usually occurred in more than one A plate row and often in the same horizontal level. In some old urchins pinched A plates were found near the peristome margin.

(3) Most deformed tests showed a clear differentiation into an almost non-deformed oral half and a highly deformed aboral half, usually inflated or otherwise displaying the aforementioned irregularities. The oral half was more symmetrical and often retained what appears to be an early ambitus (Fig. 10). Some deformed





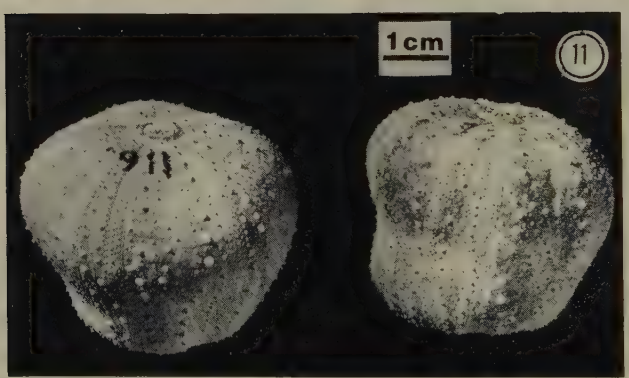


Fig. 9. A highly deformed test (H/D > 0.8), with irregularities of the ambulacral plate rows: pinching of the ambulacral system can be noted in the centre of the picture.

Fig. 10. Deformed urchins displaying an early ambitus: note that the irregular growth appears as superimposed aborally above an oral half of the test that retains the normal shape.

Fig. 11. Two deformed tests: they are aborally wider and had developed a secondary ambitus; note that the aboral half is almost perfectly regular, probably due to recovery from the preceding deformed growth; this can also be deduced from the irregularities in the transition zone (right).

sea-urchins had developed a new ambitus with a narrow transition zone, as shown by the middle urchin in Fig. 10. In the transition zone pinching of the A system was rather common.

(4) Some test shapes suggest that there may have been recovery from the deformation. The upper half of some tests showed normally arranged plates and a distinct transition zone that showed pinching and other irregularities, indicating earlier deformation (Fig. 11). The pinching, shown by older, non-deformed urchins in the peristome margin, may thus result from earlier slight deformation and recovery. In sea-urchins that show such signs of recovery the IA plate count is usually higher than in deformed urchins of equal diameter size.

(5) Deformed sea-urchins show fewer spines in the aboral region, and the spines are usually smaller.

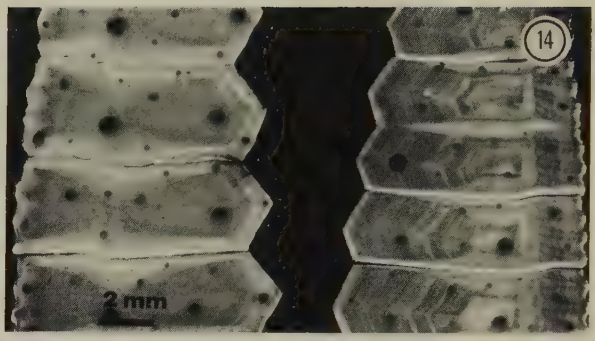
PLATE MICRO-STRUCTURES

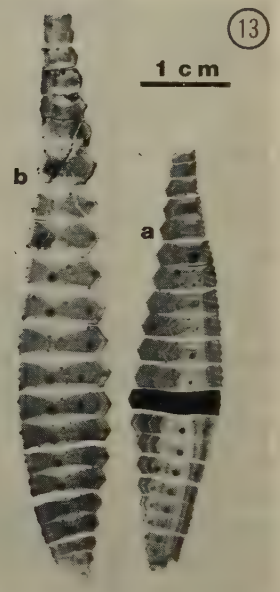
The technique of charring and immersing in xylene revealed, in transmitted light, a distinct pattern of natural concentric growth zones (Fig. 12). Jensen (1969) and Pearse & Pearse (1975) correlated such growth zones with seasons and periods of different food conditions, and found them to consist of different trabecular mesh size.

In normal *T.* cf. *gratilla* IA plate rows the growth lines form complete polygonals only in the upper plates. Below a certain level this pattern is interrupted by translucent triangles that develop in the meridional edges of each plate. The distance from a given growth line to the nearest plate edge, well represents the growth increment in that direction, since that line was formed. For each IA plate it can be seen that the maximal growth was directed towards the interradius (the middle suture of the IA row). A somewhat smaller growth rate was shown in the adradial direction (towards the ambulacral plates). A noteworthy growth gradient was seen in the meridional axis. Below the 5–8 upper IA plate level there was only a much reduced growth.

Fig. 13 shows IA plate rows of normal (Fig. 13a) and deformed (Fig. 13b) sea-urchins of almost equal diameter size. In the latter, the growth zones are less conspicuous and IA plates from below the 5–8 upper plate level were observed that show a continuous translucent growth ring surrounding the plates (Fig. 14, left). This ring pattern, that indicates equal accretion in both the meridional and the latitudinal directions, was preceded by a normal growth pattern comparable with the growth pattern of a normal urchin (Fig. 14, right). The abnormal growth pattern is seen mainly in the superambital region.

Abnormal growth in the meridional axis was also observed in the ambulacral system of deformed urchins, causing considerable distortion of the plates (Fig. 15). Growth was not limited to the border with the adjacent plates but also occurred in the demiplate sutures, which under normal conditions are not active. Such abnormal





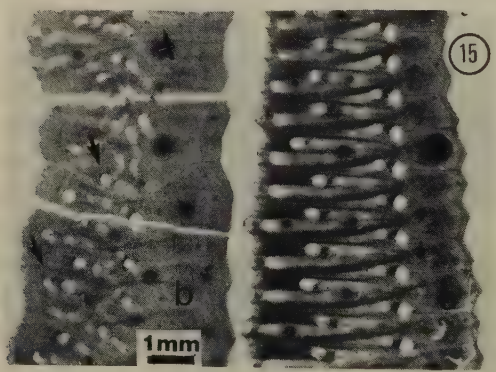


Fig. 12. Interambulacral and ambulacral plate rows of a normal *T.* cf. *gratilla* after charring and xylene immersion: the picture was taken in transmitted light and the translucent triangles and growth lines can be seen.

Fig. 13. Interambulacral plate rows of (a) normal and (b) deformed urchins with a diameter of 51 and 50 mm, respectively (upper 1–2 plates are missing): in the normal urchin meridional growth is mainly concentrated in the 5–8 upper plates while in the deformed one meridional growth zones can be detected 12–13 plates below the apex. The oral plates in both urchins are similar.

Fig. 14. Plates Nos. 11–14 of the deformed urchins and Nos. 8–12 of the normal urchins illustrated in Fig. 13 (counted downwards from the apex), are shown to illustrate the excessive growth in the meridional axis in the deformed urchin (left).

Fig. 15. Ambulacral plates of (a) normal and (b) deformed urchins: normal plates are narrow and densely packed, while deformed plates indicate uncontrolled growth along the meridional sutures; such growth can be also seen along the demiplate sutures (arrows).

growth was observed all along the A row. The oral side of the test was, however, less affected.

Discussion and Conclusions

Skeletal abnormalities in sea-urchins were often reported and described as sporadic phenomena, resulting either from genetic failures or from damage by predators and parasites. None of these factors is shown in this particular case to cause the deformations described here. The following evidence seems to point to pollution. The two deformed populations of *T.* cf. *gratilla* were inhabiting the sublittoral boulder zone on either side of the power plant, and were exposed to the same environmental disturbances created by it.

Although situated only 200 m apart, recolonization of the southern site from the northern population, after the spill, was not observed for more than 6 months. The sterile zone probably prevented the migration along the shallow beach and the movement along the sandy bottom at greater depth was also unlikely. Larval recruitment that had been observed elsewhere, evaded this site and introduction of newly settled young urchins from non-polluted localities, resulted in their total disappearance. One individual sea-urchin, however, was found to have lived in the southern site from November 1979 to January 1980, when it was collected. This single observation can prove that once adult urchins entered this area their survival was feasible.

The observation that the most affected population was situated south of the discharge point, down current, while the northern population was less affected and survived the fatal spill, points towards the effluent as the probable cause of the deformations. Some constituents of this effluent are at present being tested for harmful effects on sea-urchins.

The highly deformed urchins showed little increase in their diameter for a considerable time (Table III). Horizontal growth on the aboral side was, however, observed to form a new ambitus. In some animals this even exceeded the diameter of the lower, original ambitus. The high H/D ratio and the horizontal growth of the upper ambitus proves that growth in the deformed urchins did not stop altogether, but it was resumed with an aberrant growth pattern. The H/D ratio of deformed sea-urchins was higher for small animals and lower for larger ones. As it is known for many regular urchins, including *Tripneustes*, that growth rate is inversely related to size (Ebert, 1975) it is suggested that the degree of deformation is a function of a growth potentiality which is maximal for small animals and minimal for animals in their ultimate size. Larger urchins did not develop abnormal H/D ratio because their growth, even under normal conditions is very slow.

Allometrical data used here (Fig. 5) to demonstrate the aberrant growth pattern typical of the deformed urchins, should be interpreted with care. For normal

populations allometry is used to describe the growth pattern that is followed by the average individual. In the deformed population it displays, however, the amount of deviation from the normal proportions. Convergence of two such regression slopes, therefore, does not predict that at a certain size the deformed animal will return to the normal proportions, it only shows that animals at this size do not deviate from the normal growth pattern. The heterogeneity shown by the deformed urchins, as far as allometrical data are concerned, probably reflects individual differences in response to the pollution.

The peristome and the lantern probably continued normal growth during the deformed growth period, hence the higher peristome to diameter and lantern to diameter ratios in deformed urchins. From these observations a tentative estimate as to the time that had elapsed since the deformation started to the time of the urchins death (in the spill) can be attempted. Let us consider the two urchins illustrated in Fig. 6, with an equal peristome size (15 mm), assuming equal peristome increase for both and no horizontal growth in the deformed urchin. Thus, the deformed urchin (Fig. 6a), maintains the common diameter at the beginning of the deformation (27.5 mm), while the normal urchin (Fig. 6b) displays the final diameter, that the deformed urchin failed to reach (39 mm). Hence, the elapsed time corresponds roughly to a normal urchin's growth period between 27.5–39 mm. A diameter increase of that amount is reached by *T.* cf. *gratilla* in 2–3 months (Dafni, in prep.). Although merely speculative, this estimate was later confirmed by direct field observations. On 18 November, 1979, only one living individual, a non-deformed albinistic sea-urchin, was seen at the southern site. On 15 January, 1980, almost 2 months later, the same individual was recaptured, and was found to be deformed, with a H/D ratio of 0.88.

A problem, raised by the wider peristome of the deformed urchin, is whether peristome expansion is achieved through plate resorption or by relative displacement of the plates near its margin. In the literature this problem is unsolved and there are contradictory observations for different sea-urchin species. Lovén (1871, 1875) for *Strongylocentrotus droebachiensis* and Deutler (1926) for *Echinus esculentus*, showed that some plates are eliminated by resorption. The same was observed for cidarid urchins by Agassiz (1904) and Cutress (1965). On the other hand, in *Arbacia* there is no A plate resorption and negligible resorption of IA plates (Märkel, 1976). Moreover, Kobayashi & Taki (1969) demonstrated by tetracycline labelling of *Strongylocentrotus intermedius*, visible in UV light, that slow latitudinal growth continues even in plates on the oral side.

As the peristome growth of deformed *T.* cf. *gratilla* is not accompanied by diameter increase, it should be attained either by substantial resorption at the peristome margin or by downwards bulging of the peristome. Such bulging was observed in many individuals, but others still maintained their flat oral area, thus suggesting resorption. Their lower IA plate count (Fig. 7) may, therefore, result either from excessive resorption at the oral area or from reduced plate addition at

the apex. As no conclusive answer to this problem can yet be offered, neither possibility should be rejected.

According to Raup's model (Raup, 1968) the echinoid test growth can be described as a result of two main factors, new plate addition rate and meridional growth gradient. Normal *Tripneustes* growth conforms well with this model, but observations show that minor changes in the H/D ratio may result from partial independence between growth factors. Normal urchins, >70 mm show a high number of IA plates, compared with size (Fig. 7). This may result from continuous plate addition, even after the ultimate size has been reached. It appears that growth at this stage is limited to the aboral region, and this probably accounts for the somewhat higher H/D ratio of larger urchins (see Fig. 4). Higher H/D ratios for larger sea-urchins were also reported by Ernst (1973) for *Arbacia*.

In addition, Raup showed that most echinoid skeletal plates are shaped in patterns typical of "close-packing" due to probable stress that exists between them and the neighbouring plates. He also suggested "that growth occurs around the periphery (of the plate) and is concentrated in those regions where pressure counter to this growth is lowest". He also concluded that the "growth gradient is a result of change of stress inhibiting growth".

It has been shown (Fig. 12), that IA plates shape in *T.* cf. *gratilla* varies from the almost square shape, of the uppermost plates, to the compressed appearance they assume near the ambitus. While a considerable increase occurs in the latitudinal direction, in the meridional direction their growth is very much reduced. The A plates also change their proportions and are strongly modified during their downwards shift. The plates that are complex and are composed of one primary plate and two smaller demiplates blend together. The demiplate's sutures, that are easily visible in the uppermost plates, disappear. Märkel (1975) showed by tetracycline labelling in *Paracentrotus lividus*, that once the demiplates are united to the primary plate, they grow as one unit. All these observations, of differential latitudinal meridional growth, and the compressed appearance of the plates at the lower part of the plates' row, agree with Raup's (1968) observations. Moreover, they suggest that if the growth inhibiting stress exists it should act mainly in the meridional direction.

There are some elastic or contractile elements, which may provide the mechanical force needed to cause such meridional directed stress. These elements are (a) the lantern retractor muscles, that pull the plate rows downwards and (b) the ambulacral system with the tube feet, that exert a constant pull towards the substratum. The collagenous fibres, that bind the plates together (Travis *et al.*, 1967; Kobayashi & Taki, 1969) may act as distributors of the stress, thus forming a stress gradient along the row. The inverse proportionality between calcification and concentration of these fibres (at a certain plate edge) has already been noted (Moss & Meehan, 1967).

The exaggerated growth of deformed urchins' plates in the meridional direction

(Figs. 13–15) indicates a departure from the controlled growth that is typical of normal *Tripneustes*. It is reasonable to assume that the pollutant, possibly one of the heavy metal ions or chemicals that are emitted in the effluent, affected the balanced growth by allowing growth in directions that are otherwise inhibited. It is tempting to conclude that the deformation results either from formation of an inner pressure that causes the expansion of the test against the alleged stress balance or from damage to the elasticity or mechanical properties of the above-mentioned elements. *T.* cf. *gratilla*, because of its rapid growth, easy manipulation and sensitivity to pollution is a suitable organism to test the growth regulation through stress hypothesis and the effects of environmental factors on it.

Acknowledgements

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UPTAKE OF GLYCINE AND RELEASE OF PRIMARY AMINES BY THE POLYCHAETE *NEREIS VIRENS* (Sars) AND THE MUD SNAIL *HYDROBIA NEGLECTA* Muus

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Abstract: Uptake of glycine and release of primary amines by the polychaete *Nereis virens* (Sars) and the mud snail *Hydrobia neglecta* Muus was investigated. In a flow system, *Nereis virens* had a net influx of glycine of 32 to 39 nmol · g^{-1} fresh wt · h from a 5-μM solution of glycine. Simultaneously, an efflux of primary amines of 14 nmol · g^{-1} · h^{-1} was measured. The experiments indicated that glycine uptake and release of primary amines are two independent processes. Glycine was absorbed by *Hydrobia neglecta* from 50-μM solutions at an influx of 175 nmol · g^{-1} organic wt · h^{-1}, and simultaneously, there was an independent efflux of 25 nmol primary amine · g^{-1} · h^{-1}. In the experiments, released primary amines from both species increased the ambient concentration of primary amines, but after various periods of time these concentrations apparently stabilized. Finally, the significance of micro-organisms in amino-acid uptake experiments is considered.

INTRODUCTION

Epidermal uptake of small organic molecules by marine invertebrates has been demonstrated by several authors, as reviewed by Jørgensen (1976). Recently, much attention has been paid to uptake of free amino acids (FAA) by benthic invertebrates, e.g. glycine by the bivalve *Rangia cuneata* (Anderson, 1975), alanine by the sea urchin *Paracentrotus lividus* (Burgh, 1978), primary amines by the polychaete *Nereis diversicolor* (Stephens, 1975) and the sand dollar *Dendraster excentricus* (Stephens *et al.*, 1978), and various amino acids by the oligochaete *Enchytraeus albidus* (Siebers, 1976; Siebers & Bulnheim, 1977). Kinetic studies have shown that invertebrates absorb amino acids through mediated processes with characteristic transport constants (K_m and V_{max}) for specific amino acids (Jørgensen, 1976).

An energetic benefit from this amino-acid uptake has been questioned by Johannes & Webb (1970) who claim that efflux of amino acids from marine invertebrates exceeds influx at natural concentrations of FAA. This was demonstrated in the polychaete *Clymenella torquata* which absorbed glycine from a 1.7-μm solution. Release of free amino acids was also observed from the flatworm *Bdelloura* and the planarian *Dugesia*. Generally, according to Pandian (1975), nitrogenous waste from marine invertebrates is known to include amino acids.

In nature, the concentration of FAA in marine waters amounts to 10^{-6} M, and in marine sediments from 10^{-5} to 10^{-4} M FAA are reported (Stephens, 1972;

Jørgensen *et al.*, 1980). In order to estimate a net flux of amino acids, uptake rates at natural concentrations of FAA must be compared with release rates. Thus, in the sediment-dwelling polychaete, *Stauronereis*, the concentration in which influx equaled efflux ranged from 0.3 to 4% of the natural concentrations of FAA, suggesting that this worm obtains a net benefit from absorption of amino acids in situ (Stephens, 1972). Ferguson (1971) also reports a net uptake of amino acids into various starfishes from 37.5 μM solutions of L-alanine.

Uptake of [^{14}C]amino acids by *Mytilus californianus* and *Dendraster excentricus* was compared with removal of primary amines (fluorescamine positive material) from the incubation media by Wright & Stephens (1977, 1978) and by Stephens *et al.* (1978). In all cases a net influx of amino acids was demonstrated, even from concentrations of 1 μM or less. The concentration of primary amines tended to stabilize at 5 to 7 μM, suggesting that a balance between uptake and release was established. If long incubation times were used, however, uptake of released primary amines by bacteria may change such a balance.

In the present study, long-term experiments with uptake of glycine are presented for the polychaete *Nereis virens* (Sars) and the mud snail *Hydrobia neglecta* Muus. A flow-cell with a continuous supply of glycine is used for simultaneously monitoring of glycine uptake and release of primary amines by *Nereis virens*. Influence of micro-organisms on uptake of glycine by *Hydrobia neglecta* is investigated, as uptake of glycine by shell epigrowth and naturally occurring algae and bacteria in sea water is compared with uptake by the snails during a 4-day experiment. Release of primary amines by *H. neglecta* is also determined.

Materials and Methods

Animals and incubation media

Uptake experiments were done with two species of benthic intertidal invertebrates, *Nereis virens* and *Hydrobia neglecta* both of which were obtained from Kysing Fjord, a small estuary south of Aarhus, Denmark. For the uptake experiments, natural sea water, unfiltered or filtered through Millipore filters (pore size 0.45 μm), from the sampling locality, was used. Prior to experiments, the sea water was stored 5 to 6 wk in the dark to reduce the concentration of free amino acids.

Uptake experiments with Nereis virens

Long-term uptake of glycine by *Nereis virens* was investigated in a flow system. A Plexiglas tube (25 mm i.d., 150 mm length) provided with two stoppers and 10 small glass tubes was used as a flow-cell (Fig. 1). The internal glass tubes proved to be efficient burrows for the worms. Solutions of 5 μM of glycine were applied to this cell from a reservoir by means of a peristaltic pump with a continuous flow

of 21 ml · h^{-1}. Excurrent water was collected by a fraction collector provided with 100 or 200 6-ml test tubes. Before the start of the experiments, a drop of 5N HCl was added to the test tubes to inhibit bacterial activity. Uptake of glycine was measured using [U-^{14}C]glycine from The Radiochemical Centre, Amersham, England, as a

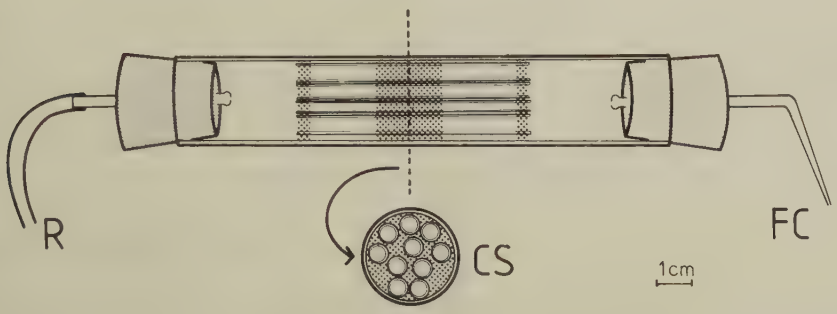


Fig. 1. Flow-cell used in long-term uptake experiments with *Nereis virens:* 10 small glass tubes were placed in the cell by means of silicone rubber, as shown in the cross-section (CS): glycine medium was applied from a reservoir (R), and used medium was collected by a fraction collector (FC).

tracer. In all cases a net uptake was measured by difference in concentration of the medium, taking samples of 0.2 ml at the input and the output of the flow system. After removing carbon dioxide by acidification, radioactivity of the samples was determined by liquid scintillation counting in Insta-Gel. Quenching was corrected using external and internal standards.

Release of primary amines

Simultaneously with uptake of glycine in the flow system, release of primary amines from the animals was measured. Fluorescamine which is a specific reagent for primary amines (e.g. amino acids) was used to estimate the primary amines, according to North (1975). Samples of 0.5 ml were taken at the input and the output of the flow-cell. Fluorescence of the fluorescamine derivatives was measured using an Aminco Fluorocolorimeter with an excitation wavelength of 390 nm and an emission wavelength of 480 nm. Glycine was used as a standard and concentrations of primary amines are given as glycine equivalents.

Ammonia excreted by the animals was observed to interfere with the primary amine measurements. This effect was pronounced when low (1 to 10 μM) concentrations of primary amines were measured in high (>10 μM) concentrations of ammonia. The fluorescence increased with increasing amounts of ammonia, but if glycine was added to the ammonia solutions, the increase in fluorescence due to ammonia was reduced (Table I). In order to determine a correction for the fluorescence by ammonia, the following equations were calculated from the results in Table I.

TABLE I

Influence of ammonia on glycine measurements as determined by fluorescamine: solutions of ammonia were prepared from reagent grade ammonium chloride; concentrations of fluorescent material given in "fluorescamine units", 1 unit is equivalent to a 1-μM solution of glycine; glycine and ammonium chloride were dissolved in artificial sea water (23‰ *S*); results as means ± SD ($n = 3$).

Concentrations of glycine (μM)	Concentrations of ammonia (μM)				
	0	25	50	75	100
0	0 ±0.21	0.15 ±0.02	0.49 ±0.06	0.74 ±0.04	1.07 ±0.07
2.5	2.40 ±0.18	3.24 ±0.21	2.98 ±0.07	3.37 ±0.16	4.63 ±0.06
5.0	5.38 ±0.06	5.40 ±0.29	5.64 ±0.31	6.28 ±0.08	6.25 ±0.09
7.5	7.54 ±0.14	8.04 ±0.16	7.95 ±0.21	8.30 ±0.03	8.51 ±0.08
10.0	10.00 ±0.43	10.14 ±0.29	10.14 ±0.17	10.55 ±0.09	10.59 ±0.14

From all measurements, the increments in fluorescence from ammonia (F_1) were empirically described by

$$F_1(x) = 0.012x + 0.064 \; (r = 0.98), \tag{1}$$

where x is the concentration of ammonia in μM.

This increased fluorescence was, however, reduced by increasing concentrations of glycine, described by F_2:

$$F_2(z) = \frac{5.30}{z} \Big/ 0.37 \; (r = 0.97), \tag{2}$$

where z is the concentration of glycine in μM. Both F_1 and F_2 are expressed in fluorescent units (1 unit = 1 μM of glycine). For $z > 2$ μM, $F_1 \cdot F_2$ was found to give appropriate corrections for the fluorescence from ammonia. In all uptake experiments, the concentration of ammonia was measured and $F_1 \cdot F_2$ was subtracted from the fluorescence measurements.

Concentrations of ammonia were measured in 0.5-ml samples of the medium, according to Solòrzano (1969), using a Chem-Lab Autoanalyzer.

Counting of bacteria

To investigate the importance of bacteria in the uptake experiments, the number of bacteria in the medium at the input and the output of the flow system was

determined according to Hobbie *et al.* (1977), using acridine orange direct counts under epifluorescence at 1000 × magnification.

Non-flow experiments with Nereis virens

A set of uptake experiments was performed with *N. virens* in 50-ml beakers containing 25-ml glycine solutions. The beakers were provided with 4-mm glass beads to replace natural sediment, as these allowed the worms to hide from each other. Samples of the medium were taken at regular intervals to measure uptake of glycine (^{14}C tracer) (0.2-ml samples), concentration of primary amines (0.5-ml samples), and ammonia (0.5-ml samples). At the end of the incubation period, the number of bacteria was determined as mentioned above.

Uptake experiments with Hydrobia neglecta

Groups of 30 *H. neglecta* were incubated in small Petri dishes in 10 ml of 50-μM glycine solutions, using filtered or unfiltered sea water. Similarly, 30 empty, fresh shells and 30 empty, dried shells of *H. neglecta* were incubated in 50 μM glycine. Concentrations of glycine (^{14}C tracer), and primary amines were measured as mentioned above. Ammonia was not measured in these experiments. Penicillin was added to some of the Petri dishes, to give final concentrations of 10^6 IU $\cdot$ l^{-1}. Streptomycin which is more inhibitory to marine bacteria (Kinne, 1977) was not used, as it interferes with fluorescamine measurements. After 4 days, the number of bacteria in the media of all the Petri dishes was counted.

Results

Uptake of glycine by *Nereis virens*

Experiments with flow system

Uptake of 5 μM glycine and release of primary amines by groups of 10 *N. virens* was investigated during 24-h experiments. In Fig. 2 a typical uptake experiment with two groups of *N. virens* is shown. Initially, a rather high release of primary amines was observed from the 2634-mg group of worms, but after 2 h, uptake of [^{14}C]glycine and occurrence of total primary amines stabilized. As shown in Table II, the 2634-mg worms reduced the concentration of [^{14}C]glycine to 0.93 μM, compared with the 1.67-μM level of the 1814-mg worms, but the highest concentration of primary amines was in the sea water from the 2634-mg worms. This relation between the weight of worms and the occurrence of primary amines may indicate that the primary amines originate from the animals. The total concentration of primary amines includes added glycine, naturally occurring primary amines, and primary amines released by the worms. Assuming that uptake of natural primary amines by

the worms is insignificant (see p. 295), efflux of primary amines from *N. virens* can be estimated, as the concentration of naturally primary amines and glycine is known. In Table II release rates are calculated under these assumptions. The released primary amines (as glycine equivalents) equate from 8 to 56%, on average 30%, of the absorbed glycine, indicating that there is a net influx of glycine.

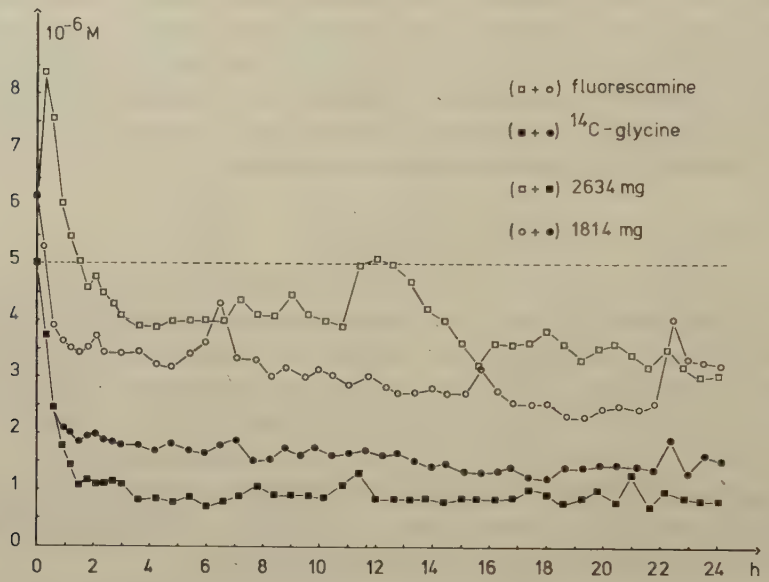


Fig. 2. Uptake of glycine and release of primary amines by two groups of 10 *Nereis virens:* natural filtered sea water containing 1.2 μM of primary amines and an initial concentration of glycine of 5 μM (^{14}C tracer) was applied to a 60 ml flow-cell at 21 ml · h^{-1}; concentration of primary amines in glycine equivalents; temperature 15 °C; 23‰ *S*; number of bacteria, initially 1.2 × 10^4 · ml^{-1}, in experiments 10.2 ± 7.1 × 10^4 · ml^{-1} (1814 mg), and 14.4 ± 15.5 × 10^4 · ml^{-1} (2634 mg); results as means.

TABLE II

Concentrations of glycine and primary amines and calculated uptake and release rates, from flow-cell experiments with *Nereis virens*, as shown in Fig. 2 (A + B) and Fig. 4 (C): release of ^{14}C calculated as glycine equivalents; results as means ± SD within the total experimental periods.

Fresh wt of animals (mg)	Concentration of [^{14}C]glycine in excurrent water (μM)	Total concentration of primary amines in excurrent water (μM) (glycine equivalents)	Uptake rates of [^{14}C]glycine (nmol · g^{-1} · h^{-1})	Release rates of primary amines (nmol · g^{-1} · h^{-1})	Release rates of ^{14}C from [^{14}C]glycine uptake (nmol · g^{-1} · h^{-1})
1814 (A)	1.67 ± 0.24	3.13 ± 0.50	39 ± 3	3 ± 6	–
2634 (B)	0.93 ± 0.16	3.92 ± 0.51	32 ± 1	14 ± 4	–
2634 (C)	–	2.85 ± 0.47	–	13 ± 4	0.58

Before the start of experiments, the sea water in the reservoir was saturated with oxygen. The oxygen consumed by the worms during the flow experiments was found to reduce the oxygen content of the water with an average 70% (data from E. Kristensen, pers. comm.). This reduction in oxygen availability does not affect uptake of amino acids or release of primary amines, but longer periods of anaerobiosis increase release of amino acids (Jørgensen & Kristensen, in prep.).

The concentration of bacteria in the flow-cell experiments increased from an initial number of 1.2×10^4 bacteria $\cdot$ ml^{-1}, to a mean of 10.2 to 14.4×10^4 bacteria $\cdot$ ml^{-1}. Since glycine medium containing the initial number of bacteria did not show any detectable reduction of glycine in 24 h, the increase of bacteria during the experiments is unlikely to cause a significant reduction of glycine and primary amines, as the turnover time of sea water in the flow-cell was below 3 h. In two other

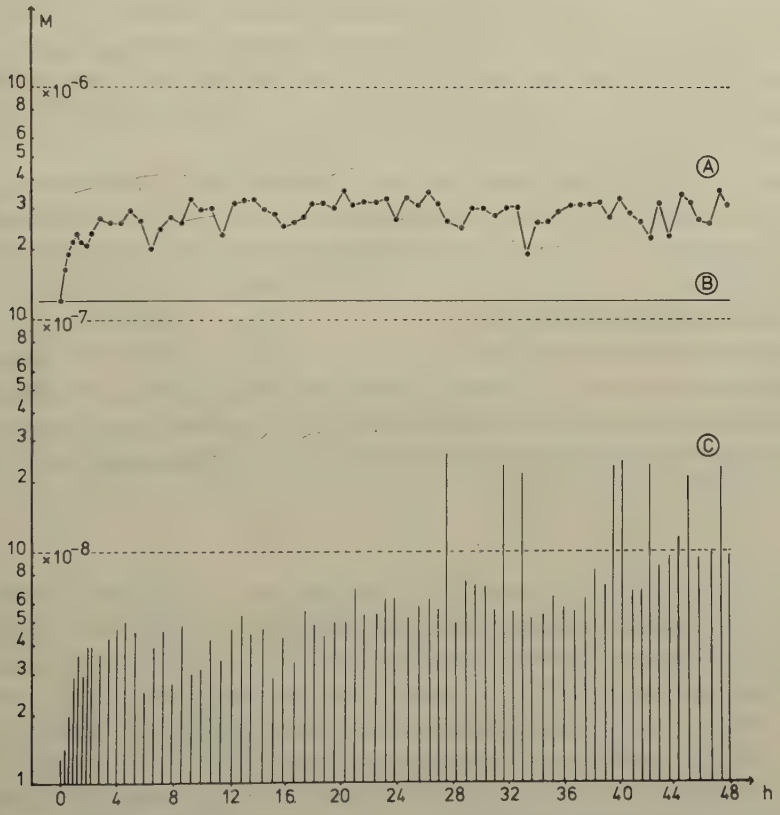


Fig. 3. Release of primary amines (A) and ^{14}C (C) by 10 *Nereis virens*: concentration of primary amines and ^{14}C in glycine equivalents; prior to experiment, the 2634-mg group of worms absorbed glycine (^{14}C tracer) as shown in Fig. 2; after a short rinse, the worms were transferred to a 60-ml flow-cell with natural filtered sea water containing 1.1 μM of primary amines (B); a flow of 21 ml $\cdot$ h^{-1} was then applied to the cell; temperature 15 °C; 23‰ *S*; number of bacteria, initially $1.2 \times 10^4 \cdot$ ml^{-1}, in experiment $11.1 \pm 3.6 \times 10^4 \cdot$ ml^{-1}; means.

experiments, however, the bacterial number exceeded 100×10^4 bacteria $\cdot$ ml^{-1}, indicating that the flow-cell may turn into a bacterial chemostat.

The uptake experiments presented in Fig. 2 showed that the concentration of [^{14}C]glycine was reduced to a level of about 1 μM. If a substantial portion of the absorbed glycine was released immediately from the worms (as [^{14}C]glycine or a ^{14}C metabolite), the actual influx of glycine would be under-estimated. In order to investigate efflux of absorbed ^{14}C material, the 2634-mg group of worms which was loaded with [^{14}C]glycine for 24 h (Fig. 2) was transferred from the flow-cell supplied with glycine, to a flow-cell with natural sea water. Before the start of the experiment, the worms were rinsed four times in filtered sea water. Release of ^{14}C material (calculated as glycine equivalents) and primary amines is shown in Fig. 3. The total concentration of primary amines increased from 1.2 μM to 2.9 ± 0.5 μM, and the concentration of ^{14}C material was equivalent to 73 ± 61 nM of glycine. If the original concentration of 1.2 μM of primary amines remained passive, the released ^{14}C material (as glycine equivalents) contributes 4% to the total primary amine release. The composition of acid non-volatile ^{14}C material is, however, unknown but the calculation indicates that only a minor portion of ^{14}C from absorbed [^{14}C]glycine is released.

During the 24-h uptake, the worms were preloaded with 981 nmole of glycine, and 48 h after transfer to the unlabelled medium, the total released acid non-volatile ^{14}C was equivalent to 74 nmole of glycine which means that 6% of the total ^{14}C material was released. As compared with uptake rates of 5 μM glycine, the release rates of ^{14}C material were very low.

As shown in Fig. 3, release of ^{14}C material increased during the experiment, suggesting that release of ^{14}C material during the 24-h uptake of [^{14}C]glycine was insignificant. Therefore, release of ^{14}C material cannot explain that glycine (measured as ^{14}C activity of the medium) was not absorbed to a level lower than 1 μM, as demonstrated in Fig. 2.

Experiments with non-flow systems

In order to examine if *N. virens* had a restricted capacity of glycine uptake from concentrations below 1 μM, groups of five worms were exposed to concentrations of [^{14}C]glycine from 78 nM to 10 μM (Fig. 4). At all concentrations, a significant uptake was observed, even from the initial concentration of 78 nM. All experiments demonstrated a similar course: from an initial rapid uptake, the uptake declined slowly; e.g. the uptake in the 1.0-μM experiment stabilized at 0.25 μM, but if another group of worms was exposed to a 0.40-μM glycine solution, a rapid uptake reappeared. It is uncertain why this decline in uptake occurs after 3 to 4 h.

The specific concentration of glycine occurring in the water was unknown, but the uptake rates (Table III) indicate that the concentration must be below 100 nM, since the rates still declined at this concentration. Ignoring the natural concentration of glycine, the glycine was reduced to a level of 6 nM in the experiment.

Release of primary amines from three groups of *N. virens* was investigated in natural sea water in a non-flow system. Fig. 5 shows that the release increased to a plateau of 3.1 μM in 7 h. If the natural primary amines in the sea water were not absorbed by the worms, the release amounts to 14 nmol · g^{-1} fresh wt · h^{-1}. This efflux is in agreement with the rates measured in the flow-cell experiments (Table II).

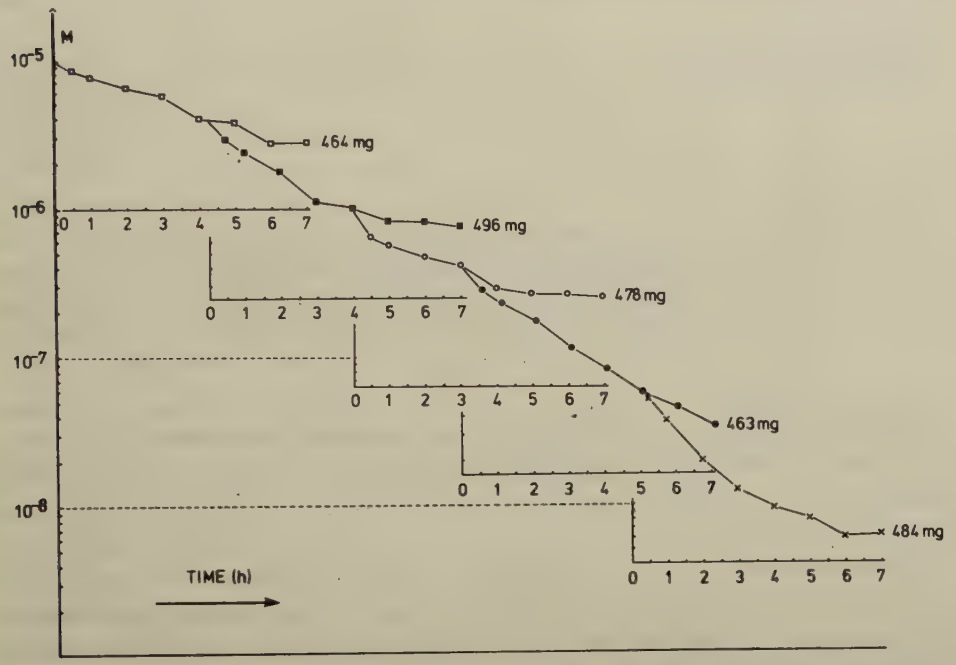


Fig. 4. Uptake of glycine (^{14}C tracer) by groups of five *Nereis virens*: uptake curve composed of five individual experiments; medium, 25 ml solutions of glycine in natural filtered sea water; weight of each group of worms indicated; temperature 15 °C; 23‰ *S*; number of bacteria (average of all experiments), initially 1.2 × 10^4 · ml^{-1}, in experiments 9.3 ± 4.6 × 10^4 · ml^{-1}; means.

TABLE III

Uptake rates of glycine by five groups of *Nereis virens*: rates are calculated from reduction of glycine during the initial 30-min incubation; see caption to Fig. 4.

Initial concentration of glycine (μM)	Uptake rates of [^{14}C]glycine (nmol · g^{-1} fresh wt · h^{-1})
10	156
4	106
1	42
0.40	13
0.168	4.0
0.078	2.6

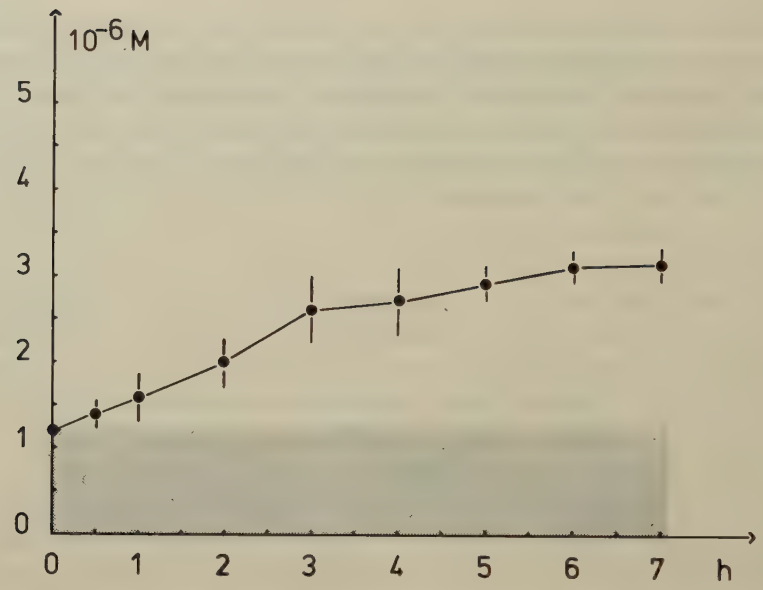


Fig. 5. Release of primary amines (glycine equivalents) by three groups of *Nereis virens* in 25 ml natural filtered sea water: dotted area indicates naturally occurring primary amines; average wt of worms 469 ± 8 mg; temperature 15 °C; 23‰ *S*; number of bacteria (average of all experiments), initially $1.2 \times 10^4 \cdot ml^{-1}$, after 7 h $9.3 \pm 4.6 \times 10^4 \cdot ml^{-1}$; means.

Release of ammonia

Ammonia is a predominant nitrogenous waste from marine invertebrates (Pandian, 1975). From the flow-cell experiment in Fig. 3, the primary amine: ammonia excretion ratio can be estimated. The concentration of ammonia in the output water was 63.4 μM, and initially the sea water contained 1.9 μM of ammonia. As the flow of the cell was 21 ml · h ^{1}, the excretion rate of ammonia was 490 nmol · g ^{1} fresh wt · $^{-1}$. Pandian (1975) suggested that most primary amines released from invertebrates are amino acids, including taurine. Thus, about 14 nmol amino acid · $g^{-1} \cdot h^{-1}$, roughly equivalent to 14 nmol amino nitrogen · $g^{-1} \cdot h^{-1}$, is released from the animals. Consequently, the release of primary amines amounts to ≈3% of the ammonia excretion.

UPTAKE OF GLYCINE BY *HYDROBIA NEGLECTA*

Influence of natural populations of bacteria and micro-organisms on uptake of amino acids by invertebrates was investigated for *H. neglecta* in a non-flow system. Simultaneously with uptake of glycine, the concentration of primary amines was measured. At the end of each experiment, the number of bacteria in the media was counted. The experiments were performed with groups of 30 snails during 4 days. Experimental media were 50-μM solutions of glycine in filtered or unfiltered natural

sea water. The filtration of sea water (0.45-μm filters) reduced the bacterial number from 4.1×10^4 to $0.7 \times 10^4 \cdot ml^{-1}$. Four sets of experiments were carried out in Petri dishes with: (1) 30 snails with natural epigrowth of green microalgae on the shells, in 50 μM glycine, (2) 30 empty, fresh shells, in 50 μM glycine, (3) 30 empty, dried (105 °C) shells which were equilibrated in filtered sea water 8 h before start of uptake of 50 μM glycine, (4) 50-μM glycine medium without shells and snails, and (5) 30 snails in sea water without glycine added. In half of the experiments, 10^6 IU penicillin $\cdot l^{-1}$ was added. Using penicillin in long-term experiments, it should be noted that the antibiotic effect declines after 2 to 3 days (Kinne, 1977), and only Gram-positive bacteria are inhibited (MacLeod, 1971).

Results from one set of experiments are shown in Fig. 6A (uptake measured with [^{14}C]glycine as a tracer), and in Fig. 6B (uptake measured with fluorescamine). A corresponding set of experiments was performed with penicillin added to the media: Fig. 6C ([^{14}C]glycine), and Fig. 6D (fluorescamine). The natural concentrations of primary amines in the sea water was 1.1 μM.

Snails

The snails reduced the concentration of [^{14}C]glycine to 1.5 μM in 24 h. Within this period, the ^{14}C and the fluorescamine measurements are rather similar, but probably due to excretion of primary amines by the animals, and to the natural concentration of primary amines, the total amount of primary amines was slightly higher than the ^{14}C measurements. It is remarkable that an initial release of primary amines by the snails (dotted area in Fig. 6B, D) in both experiments increased the concentration of primary amines to ≈ 5 μM, and that this concentration was maintained for 24 h. After 24 h, there was a small decrease of primary amines and in all experiments a level of 2 to 5 μM was measured after 4 days.

Net influx of glycine by *H. neglecta* can be estimated from Fig. 6. In 24 h, the snails reduced the total primary amines to 5 μM. Simultaneously, the empty fresh shells reduced the primary amines to 30 μM which roughly indicates that *H. neglecta* accounts for a 25-μM reduction of primary amines. If shells of the snails comprise 50% of the total fresh wt (J. Hylleberg, pers. comm.), the average net influx is 170 nmol $\cdot g^{-1}$ organic wt $\cdot h^{-1}$. Similar calculations of influx determined with [^{14}C] glycine give 195 nmol $\cdot g^{-1} \cdot h^{-1}$, suggesting that 25 nmol primary amines $\cdot g^{-1} \cdot h^{-1}$ are released. This efflux of primary amines implies that the concentration should increase by 3.5 μM in 24 h. Release of primary amines from the snails in sea water without glycine, however, increased the concentration to 5 μM within the first hour.

Fresh shells

Uptake of glycine by empty, fresh shells was probably caused by algae attached to the shells, as penicillin only had a small effect. The experiments demonstrate

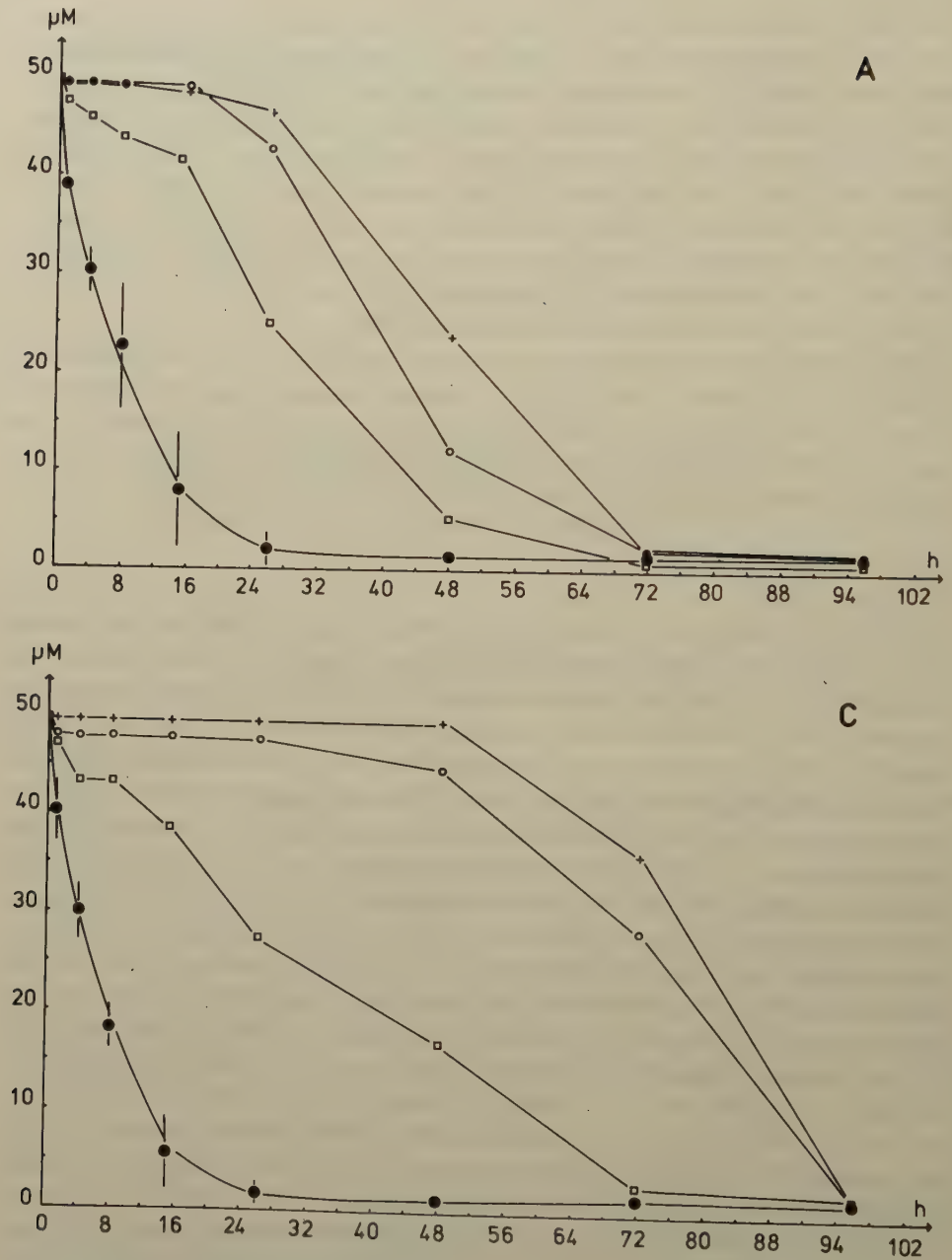
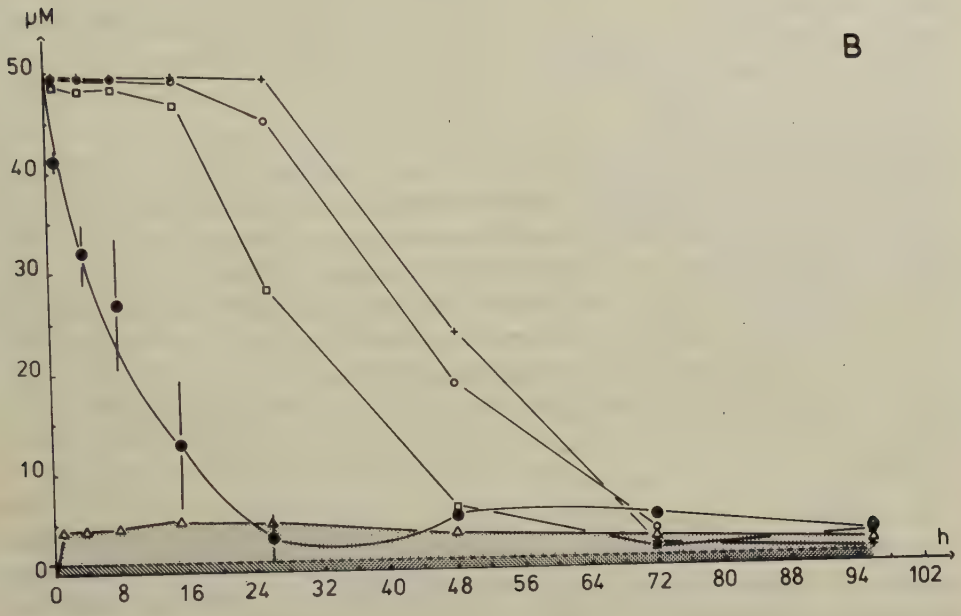
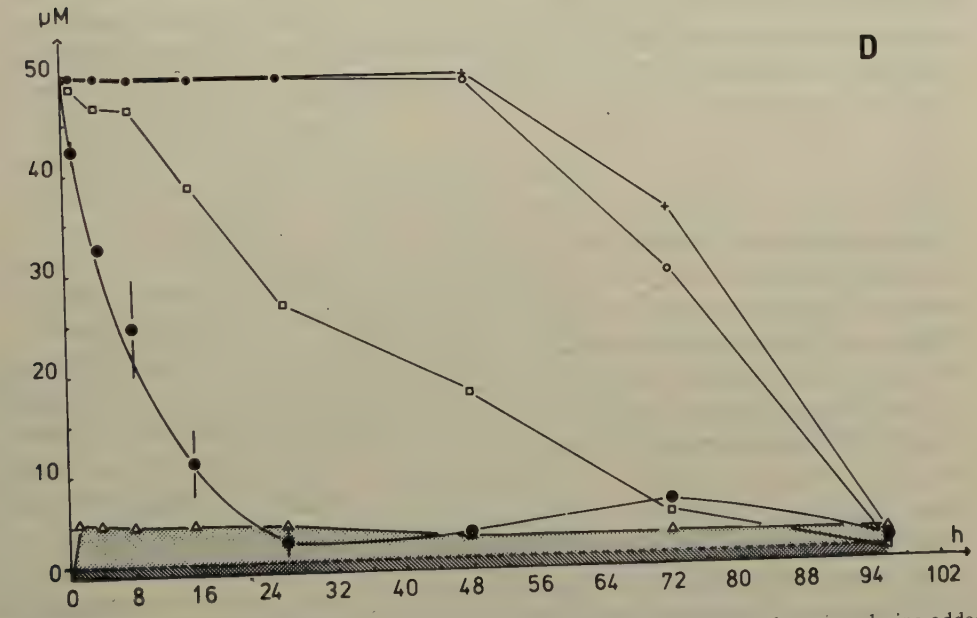


Fig. 6. Uptake experiments with *Hydrobia neglecta*: results from two sets of experiments are shown; ●, uptake by 3 × 30 *H. neglecta*, average wt of 30 snails (with shells) 103.2 ± 9.9 mg, (mean ± SD); □, uptake by 30 fresh empty shells; ○, uptake by 30 dried empty shells, equilibrated in filtered sea water 8 h before start of uptake; +, 50-µM glycine medium; medium in these experiments was 50 µM





glycine in 10 ml 23‰ *S* sea water; △, release of primary amines by 30 *H. neglecta*, no glycine added to the medium; uptake determined with ^{14}C tracer (A and C) and fluorescamine (B and D); 10^6 units of penicillin added in C and D; temperature 15 °C; 23‰ *S*; natural concentration of primary amines (1.2 µM) indicated by a dashed line.

that *H. neglecta* during the initial 24 h absorb glycine three to four times faster than the shell epigrowth.

Dried shells and pure medium

The concentration of glycine in dishes with dried shells and pure medium decreased in much the same way. Without penicillin, the concentration decreased after 24 h, and with penicillin, the 50-μM concentration was maintained until 48 h. The succeeding reduction of glycine must be due to growth of bacteria.

The two corresponding sets of experiments with unfiltered sea water demonstrated a very similar course to the experiments shown in Fig. 6. The natural concentration of micro-organisms (algae and bacteria) was apparently too small to produce a significant uptake of glycine.

Bacteria

The number of bacteria in the Petri dishes was determined using acridine orange direct counts after 4 days of incubation. As compared with the initial concentrations, the number of bacteria typically increased three to four times during the experiments. In about 10% of the Petri dishes, the concentration increased more than 20 times, whether snails were present or not. Filtration or addition of penicillin only had an insignificant effect. Correspondingly, no obvious correlation between the initial concentration of glycine and the final number of bacteria was observed.

Discussion

From an ecological point of view, an important question is whether *Nereis virens* and *Hydrobia neglecta* obtain a net influx of amino acids from natural concentrations of free amino acids. Both species are associated with the sediment. *Hydrobia* spp. are usually buried in the shallow sediment (Ankar & Jansson, 1973; Hylleberg, 1976), and *Nereis virens* is a true sediment-dweller which may penetrate to a depth of 65 cm (Muus, 1967). In Kysing Fjord where animals for the experiments were collected, the concentration of dissolved, free amino acids in the sediment ranged from 4 to 28 μM (Jørgensen *et al.*, 1980). From Jørgensen (1979a), uptake of glycine and aspartic acid by *N. virens* from natural concentrations is determined to 7 to 65 nmol $\cdot$ g^{-1} $\cdot$ h^{-1}. As release of primary amines by *N. virens* was 14 nmol $\cdot$ g^{-1} $\cdot$ h^{-1}, a net flux from uptake of natural concentrations of these two amino acids must be obtained. Considering the total concentration of amino acids in the sediment, a large net uptake of amino acids is achieved by *N. virens* in situ.

The present non-flow experiments clearly show that *N. virens* has capacity to absorb glycine from low concentrations. The observed 1-μM level in the flow experiments may be caused by (1) a minor portion of the glycine medium is passing

through the cell without being exposed to the animals, or (2) the flow of the medium may have exceeded the uptake capacity of the worms. It is difficult, however, directly to compare a flow and a non-flow system.

In natural sea water, release of primary amines by *Hydrobia neglecta* increased the ambient concentration of primary amines to about 5 μM, and when an initial concentration of 50 μM glycine was added, this 5-μM level was re-established in 24 h. This steady state suggests a balance between influx and efflux of primary amines by the snails. As compared with natural concentrations of interstitial primary amines (Jørgensen, 1979b), this steady-state concentration is considerably lower, indicating a net influx of primary amines (e.g. amino acids) in situ.

When *Nereis virens* was kept in natural sea water, the concentration of primary amines also increased, but the concentration tended to stabilize at a level of 3 μM which is comparable with the *Hydrobia neglecta* experiments. Corresponding results are reported by Wright & Stephens (1978) for *Mytilus californianus*, and by Stephens *et al.* (1978) for *Dendraster excentricus*. These steady-state concentrations must be explained from uptake of released primary amines by the animals. In the present experiments with *Nereis virens*, release of primary amines was unchanged when glycine was added the sea water. Similarly, Stephens (1968) reports that release of ^{14}C from [^{14}C]amino acids absorbed by the polychaete *Dorvillea articulata* was independent of the ambient concentration of amino acids. Diffusion of amino acids from the animals cannot explain the observed steady states, since intracellular concentrations of free amino acids in most marine invertebrates amount to 2 to 5×10^{-1} M (Wright & Stephens, 1977). This favours an equilibrium situation between efflux and efflux of primary amines.

Uptake of glycine by *Hydrobia neglecta* is complex to determine, as the shell epigrowths also absorb amino acids. The empty, fresh shells of *H. neglecta* demonstrated a significant uptake of glycine, but 3 days were required to reduce the glycine to 5 μM, while this was achieved by the snails in 24 h. As penicillin delayed reduction of glycine in the incubation medium which contained about 15% of the initial populations of micro-organisms, but not uptake of glycine by empty, fresh shells, microalgal epigrowth of the shells probably reduced the glycine. Uptake of amino acids by marine microalgae is reported by North & Stephens (1972). After 4 days, however, the number of bacteria in the Petri dishes did not allow such a conclusion, since an apparently unpredictable number of bacteria occurred. But an increase of bacteria in 4 days does not contradict an initial antibiotic effect of penicillin. The high number of bacteria in some of the *H. neglecta* experiments may have influenced the efflux–influx balance, as bacteria also absorb primary amines. This may explain the decrease of primary amines after 72 h in Fig. 6B, D.

Natural sea water for the experiments was collected in November. In winter small amounts of amino acids occur in Kysing Fjord (Jørgensen *et al.*, 1980). Thus, the concentration of free glycine in freshly collected sea water never exceeded 150 nM, and the total concentration of free amino acids made up from 13 to 57% of the total

free primary amines. Prior to the experiments, the unfiltered sea water was stored in the dark for 5 to 6 wk to reduce the concentration of easily bio-degradable organic substances like amino acids. Therefore, only a minor portion of the naturally occurring primary amines was probably amino acids. Since a residual amount of primary amines of 1.2 μM was not assimilated by bacteria, it is unlikely that these are absorbed by *Nereis virens* and *Hydrobia neglecta*.

The bulk of nitrogen excreted by marine invertebrates consists of ammonia, but release of amino acids by 21 species of marine invertebrates constituted from 2 to 36% of the total nitrogenous waste (Pandian, 1975). In long-term experiments with *Nereis virens*, release of primary amine-nitrogen amounted to 4% of the excretion of ammonia-nitrogen. This indicates that only a minor portion of the total nitrogenous excretion from this worm consists of organic nitrogen such as amino acids.

Amino acids absorbed by marine invertebrates have been proved to take part in vital processes of the animals. Immediately after uptake, the main portion of absorbed amino acids occurs as free, intracellular amino acids, but a variable portion is incorporated into proteins, lipids, and DNA, or is respired (Anderson & Bedford, 1973; Jørgensen, 1979a). Since free intracellular amino acids are active in osmoregulation of many marine invertebrates (Gilles, 1975), transepidermal absorption of amino acids may be important to animals which live in fluctuating salinities.

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